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Outcome of Puumala Hantavirus-induced Nephropathia Epidemica

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Ei ole olemassa muureja

        on vain siltoja

Ei ole olemassa suljettuja ovia

        on vain portteja

-Tommy Tabermann-

To my mother and Jari

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LIST OF ORIGINAL PUBLICATIONS

This dissertation is based on the following five original studies, which are referred to in the text by their Roman numerals I-V.

I   Miettinen M, Mäkelä S, Ala-Houhala I, Huhtala H, Kööbi T, Vaheri A, Pasternack A, Pörsti I, Mustonen J: Tubular proteinuria and glomerular filtration six years after Puumala hantavirus-induced acute interstitial nephritis. Nephron Clin Pract 2009;112:115-120.

II Miettinen M, Mäkelä S, Ala-Houhala I, Huhtala H, Hurme M, Kööbi T, Partanen J, Pasternack A, Vaheri A, Pörsti I, Mustonen J: The severity of acute Puumala hantavirus infection does not predict the long-term outcome of patients. Nephron Clin Pract 2010;116:89-94.

III   Miettinen M, Mäkelä S, Ala-Houhala I, Huhtala H, Kööbi T, Vaheri A, Pasternack A, Pörsti I, Mustonen J: Ten-year prognosis of Puumala hantavirus-induced acute interstitial nephritis. Kidney Int 2006;69:2043-2048.

IV   Mäkelä S, Jaatinen P, Miettinen M, Salmi J, Ala-Houhala I, Huhtala H, Hurme M, Partanen J, Pörsti I, Vaheri A, Mustonen J: Hormonal deficiencies during and after Puumala hantavirus infection. Eur J Clin Microbiol Infect Dis 2010;29:705-713.

V Miettinen M, Mäkelä S, Haapala M, Helanterä A, Helin H, Vänttinen T, Mustonen J: Glomerulonephritis emerging shortly after Puumala hantavirus infection: a report on 7 patients. Clin Nephrol 2011;75:550-556.

ABBREVIATIONS

ABP	ambulatory blood pressure
AIN	acute interstitial nephritis
ARDS	acute respiratory distress syndrome
BMI	body mass index
BP	blood pressure
C	complement
CMV	cytomegalo virus
CNS	central nervous system
CRP	C-reactive protein
CTL	cytotoxic T lymphocytes
CVB	Coxsackie virus B
DOBV	Dobrava virus
E2	estradiol
EBV	Epstein-Barr virus
ECG	electrocardiogram
ECHO	echocardiography
EEG	electroencephalography
EIA	enzyme immunoassay
ERPF	effective renal plasma flow
FF	filtration fraction
FSH	follicle-stimulating hormone
fT4	free thyroxine
GFR	glomerular filtration rate
GH	growth hormone
GN	glomerulonephritis
HBV	hepatitis B virus
HCV	hepatitis C virus
HCPS	hantavirus cardiopulmonary syndrome
HFRS	hemorrhagic fever with renal syndrome
HIV	human immunodeficiency virus

HLA	human leukocyte antigen
HSV	herpes simplex virus
HTNV	Hantaan virus
IDO	indoleamine 2,3-dioxygenase
IF	immunofluorescence
IFG	impaired fasting glucose
IFN	interferon
Ig	immunoglobulin
IgAN	immunoglobulin A nephropathy
IL	interleukin
LH	luteinizing hormone
MesGN	mesangial glomerulonephritis
MGN	membranous glomerulonephritis
MPGN	membranoproliferative glomerulonephritis
MRI	magnetic resonance imaging
NE	nephropathia epidemica
NO	nitric oxide
NP	nucleocapsid protein
PCR	polymerase chain reaction
PRL	prolactine
PUUV	Puumala virus
PV B19	parvovirus B 19
RNA	ribonucleic acid
SAAV	Saaremaa virus
SD	standard deviation
sIL-2R	soluble interleukin-2 receptor
SNV	Sin Nombre virus
Testo	testosterone
TLR	toll-like receptor
TNF	tumor necrosis factor
TPOAb	thyroid peroxidase antibodies
TSH	thyrotropin
WHO	World Health Organization

ABSTRACT

Nephropathia epidemica (NE), a mild type of hemorrhagic fever of renal syndrome (HFRS), is caused by Puumala hantavirus (PUUV). A sudden onset of high fever, headache, nausea and back and abdominal pain are the most typical symptoms. Renal involvement comprises oliguria followed by polyuria together with proteinuria and hematuria. Renal histopathology reveals acute tubulointerstitial nephritis. A complete recovery is the usual outcome.

The long-term prognosis of NE has previously been assessed in a retrospective study of 46 patients with previous NE (Mäkelä et al. 2000). Three to seven years after serologically confirmed PUUV infection, patients evinced a higher glomerular filtration rate (GFR), more proteinuria and higher ambulatory blood pressure (ABP) than healthy, seronegative controls (Mäkelä et al. 2000). This cohort of NE patients and controls were re-examined a median of 10 years after acute NE. Thirty-six patients and 29 controls took part. There were no longer significant differences in GFR and ABP or in the amount of proteinuria between patients and controls. There was nevertheless a slight predominance of hypertensive subjects in the patient group.

In the present series, the renal outcome and blood pressure (BP) of patients with previous NE were further studied in a prospectively collected group of 37 patients. Six years after acute NE, the patients had higher GFR, more proteinuria and higher ABP compared with 38 seronegative controls. The clinical severity of the acute phase of NE did not influence the long-term findings.

Serum hormonal levels were studied in 54 patients during acute NE, after 3 months, and after 1 and 5 years. All levels were analyzed retrospectively. During

acute NE, 56 % of the patients evinced hormonal abnormalities. Chronic, overt hormonal deficits were detected in 17 % of the patients after a median follow-up of 5 years: 5 had hypopituitarism and 5 had primary hypothyroidism. Furthermore, in 5 males chronic subclinical testicular failure was diagnosed. The acute-phase central hormonal deficiencies correlated with the severity of the acute renal failure and inflammatory markers. In contrast, the severity of acute NE was not associated with the development of chronic hormonal deficiencies.

The clinical courses of seven patients with nephrotic-range proteinuria concomitant with hematuria emerging 1 to 12 weeks after acute NE have been described. In five cases a renal biopsy specimen showed membranoproliferative glomerulonephritis (MPGN), while membranous glomerulonephritis (MGN) and mesangial glomerulonephritis (MesGN) were detected each in one. All patients achieved remission in a median of 0.6 years, indicating a good prognosis.

In conclusion, the present series confirms the favorable long-term outcome of acute NE. However, the possibility remains that NE may predispose patients to hypertension. Hormonal alterations are common during and after acute NE. Furthermore, as a rare phenomenon, the convalescent phase of acute NE may be complicated by glomerulonephritis.

TIIVISTELMÄ

Myyräkuume on munuaisoireinen verenvuotokuume, jonka aiheuttaa hantavirusten ryhmään kuuluva Puumala-virus. Tavanomaisia oireita ovat äkillinen korkea kuume, päänsärky, pahoinvointi sekä selkä- ja vatsakivut. Munuaisvaurioon liittyvät ohimenevä oliguria ja sitä seuraava polyuria, ja virtsanäytteessä nähdään protein- ja hematuriaa. Munuaisvaurion vaikeusaste vaihtelee, ja vain pieni osa potilaista tarvitsee dialyysihoitoa. Munuaisen histopatologisessa tutkimuksessa todetaan äkillinen tubulointerstitiaalinen nefriitti. Myyräkuumeen ennustetta on pidetty hyvänä.

Taudin pitkäaikaisennustetta on aiemmin tutkittu retrospektiivisesti kerätyssä 46 potilaan aineistossa. Tutkimuksessa havaittiin, että 3-7 vuotta akuutin vaiheen jälkeen myyräkuumeen sairastaneilla oli korkeampi glomerulusten suodatusnopeus (GFR) 51CrEDTA-menetelmällä mitattuna, enemmän proteinuriaa sekä korkeampi systolinen verenpaine vuorokausimittauksessa kuin terveillä verrokeilla.

Tässä väitöskirjatyössä kutsuttiin em. retrospektiivisesti kerätyn aineiston potilaat ja verrokit uudestaan tutkimuksiin, kun potilaiden myyräkuumeen sairastamisesta oli kulunut keskimäärin 10 vuotta. Havaittiin, että 36 potilaan ja 29 verrokin välillä ei ollut enää eroa GFR:ssa, proteinurian määrässä tai verenpaineen vuorokausimittauksen tuloksissa. Kuitenkin näytti siltä, että potilasryhmässä oli enemmän kohonnutta verenpainetta sairastavia kuin verrokkiryhmässä, kun otettiin huomioon verenpaineen vuorokausirekisteröinnin lisäksi verenpainelääkkeiden käyttö sekä vuorokausirekisteröintiin osallistumattomien potilaiden vastaanotolla mitattu verenpaine.

Munuaisten toimintaa ja verenpainetta tutkittiin myös 37 prospektiivisesti kerätyn potilaan aineistossa 6 vuotta akuutin taudin jälkeen, ja tuloksia verrattiin 38 seronegatiiviseen verrokkiin. Potilailla todettiin retrospektiivistä tutkimusta vastaavasti korkeampi GFR ja verenpaine sekä enemmän proteinuriaa kuin verrokeilla. Myyräkuumeen akuutin vaiheen taudin vaikeusaste ei vaikuttanut pitkäaikaislöydöksiin.

Viidenkymmenenneljän myyräkuumepotilaan aineistossa tutkittiin aivolisäke-, sukupuoli- ja kilpirauhashormonien seerumipitoisuuksia taudin akuutissa vaiheessa, 3 kuukauden, sekä 1 ja 5 vuoden kuluttua tautiin sairastumisesta. Hormonipitoisuudet analysoitiin jälkikäteen. Myyräkuumeen akuutissa vaiheessa 56 %:lla potilaista todettiin poikkeavuuksia seerumin hormonipitoisuuksissa. Kroonisia poikkeavuuksia todettiin puolestaan 17 %:lla potilaista 5 vuoden seurannan jälkeen: 5 potilaalla aivolisäkkeen vajaatoiminta ja 5 potilaalla primaarinen kilpirauhasen vajaatoiminta. Subkliininen kivesten vajaatoiminta diagnosoitiin lisäksi 5 miespotilaalla. Myyräkuumeen akuutin vaiheen sentraaliset hormonipuutokset korreloivat akuutin munuaisten vajaatoiminnan vaikeusasteen sekä tulehdusta kuvaavien muuttujien kanssa. Sen sijaan krooniset hormonipoikkeavuudet eivät näyttäneet olevan yhteydessä myyräkuumeen vaikeusasteeseen.

Väitöskirjassa kuvataan myös 7 potilasta, joilla todettiin nefrootinen oireyhtymä ja hematuria 1-12 viikkoa myyräkuumeen akuutin vaiheen jälkeen. Viidellä oli munuaisbiopsiassa löydöksenä membranoproliferatiivinen, yhdellä membranoosi ja yhdellä mesangiaalinen glomerulonefriitti. Kaikki potilaat saavuttivat remission keskimäärin 0.6 vuoden aikana viitaten hyvään ennusteeseen.

Väitöskirja vahvistaa aiempaa käsitystä myyräkuumeen hyvästä pitkäaikaisennusteesta. Myyräkuume saattaa kuitenkin altistaa joitakin potilaita

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verenpaineen kohoamiselle. Seerumin hormonipitoisuuksien poikkeavuudet ovat tavallisia taudin akuutissa vaiheessa, ja osalla potilaista havaitaan hormonimuutoksia vuosien kuluttua sairastumisesta. Myyräkuumeen mahdollisia pitkäaikaishaittoja ei voida ennustaa taudin akuutin vaiheen vaikeusasteen perusteella. Myyräkuumeen toipumisvaiheeseen voi myös harvinaisissa tapauksissa liittyä glomerulonefriitti, jonka ennuste vaikuttaa suotuisalta.

1. INTRODUCTION

Nephropathia epidemica (NE) is a zoonosis belonging to the group of hemorrhagic fever with renal syndrome (HFRS). HFRS drew significant attention in the 1950s, when United Nations soldiers from the Korean War suffered from an acute illness later known to be caused by Hantaan virus (HTNV) (Smadel 1953, Lee et al. 1978). In 1980 NE was reported to be caused by Puumala virus (PUUV) carried by bank voles (*Myodes glareolus*) (Brummer-Korvenkontio et al. 1980). Several other hantaviruses causing human disease have since been found. In the Americas, a severe hantavirus infection with high mortality was described in the 1990s (Nichol et al. 1993), and subsequently named hantavirus cardiopulmonary syndrome (HCPS).

The clinical picture of acute NE is characterized by a sudden onset of fever with headache, nausea and back and abdominal pain together with acute renal failure and thrombocytopenia accompanied by hemorrhagic manifestations (Lähdevirta 1971, Settergren et al. 1989, Mustonen et al. 1994a, Braun et al. 2010). Renal histopathology reveals acute tubulointerstitial nephritis (AIN) (Mustonen et al. 1994b). The clinical course varies from subclinical to fatal, although mortality is rare (Vapalahti et al. 2003).

The long-term prognosis of NE is considered to be favorable. However, previous studies have suggested changes in renal function or proteinuria up to 6 years (Lähdevirta 1971, Lähdevirta et al. 1978, Settergren et al. 1989, Settergren et al. 1990, Elisaf et al. 1993, Mäkelä et al. 2000, Ala-Houhala et al. 2002), and elevated

blood pressure (BP) up to 5 years after acute NE (Mäkelä et al. 2000). Furthermore, NE may be complicated by pituitary hormonal disturbances (Saltevo and Forslund 1992, Settergren et al. 1992, Hautala et al. 2002, Sane and Färkkilä 2002, Pekic et al. 2005) or consequent glomerulonephritis (GN) (Grcevska et al. 1990, Mustonen et al. 2001).

Between the years 1990 and 2006 a total of 34 000 hantavirus infections were confirmed in Europe, and 70 % of them were reported from Finland as being caused by PUUV (Heyman and Vaheri 2008). The number of hantavirus infections diagnosed in Europe has recently been on the increase (Heyman and Vaheri 2008). Wide awareness of the disease and better diagnostics are obvious explanations for this. However, climate change may contribute to the problem in that in Northern Europe milder winters and thinner snow cover have probably led to the higher incidences of NE during the last few years (Pettersson et al. 2008a, Evander and Ahlm 2009). Further, in Western Europe the increased incidences may be explained by higher summer temperatures, offering more nourishment for bank voles (Tersago et al. 2009).

The present study was undertaken to examine the long-term outcome of NE in respect of renal function, proteinuria and BP. Hormonal changes during and after acute disease were also assessed. Further, the study describes seven patients with glomerulonephritis shortly after acute NE.

2. REVIEW OF THE LITERATURE

2.1 Hantaviruses and hantavirus infections

2.1.1 History

In Finland in 1926 Theodor Waldemar Tallqvist recognized a clinical syndrome resembling acute NE in the southern part of the country (Forsius 1995). NE itself, however, was first described in 1934 independently by two Swedish physicians (Myhrman 1934, Zetterholm 1934), the first case being observed in 1933 in north-central Sweden. The clinical picture of the "new" disease was an acute onset of fever with chills, malaise, vomiting, headache, and back and abdominal pain. Renal syndrome was characterized by heavy proteinuria with oliguria and azotemia. The disease had a good prognosis. Subsequently the investigators Stuhlfauth from Germany and Hortling from Finland described a similar epidemic disease occurring among German and Finnish soldiers in northern Finland during World War II, this being also reported by Fredrik Saltzman in 1945 (Forsius 1995). The histopathology of the renal manifestation of acute NE was first described in Finland by Kuhlbäck and co-workers (1964). The term "Nephropathia epidemica" was suggested by Myhrman in 1945, although he himself also named the condition Myhrman's disease (Forsius 1995). In Finland, "myyräkuume" was proposed as the Finnish name for this disease in 1980s (Lähdevirta and Savola 1985).

As far back as the first millennium (Lee 1982a) and the Middle Ages (Bridson 2001), however, there are descriptions of HFRS-like diseases. Furthermore, it has also been suggested that hantavirus disease may have appeared during the American civil war in the 1860s and during World War I in 1915 and 1916, as well as in Vladivostok regional hospital in 1913–1914 (Heyman et al. 2009). However, it was not until the Korean War in 1951–1954 that HFRS became better known to Western medicine. More than 3000 United Nations soldiers near the small Hantaan River suffered from an acute febrile disease with nephritis, mortality rising to 7 % (Smadel 1953, Johnson 2001). It was this epidemic which gave impulse to more extensive research into this disease.

In the past HFRS went under many names, until a working group on HFRS in the World Health Organization (WHO) meeting in Tokyo in 1982 recommended the current term (Lee 1982a). In the Soviet Union the condition had been named hemorrhagic nephroso-nephritis or HFRS, in China Songo fever, in Scandinavia NE and in Eastern Europe epidemic nephritis (Lee 1982a). The term epidemic hemorrhagic fever was also used in Japan, Eastern Europe and China (Lee 1982a).

2.1.2   Virology

As far back as 1953 Gajdusek suggested a relationship of NE to Korean hemorrhagic fever, and the possible viral etiology of these diseases was first proposed first in 1960s (Gajdusek 1962). However, it was not until 1976 that Lee and co-workers identified the causative agent of Korean hemorrhagic fever, currently named Hantaan virus (Lee et al. 1978). It was isolated near the Hantaan River in the lungs of striped field mice (*Apodemus agrarius*). A few years later the

causative agent of NE was found in the lungs of bank voles in Puumala, Finland, and named Puumala hantavirus (Brummer-Korvenkontio et al. 1980).

Up to now, over 40 different hantavirus species belonging to the family *Bunyaviridae* have been found almost all over the world (Heyman et al. 2009), and 20 of them have so far been described as being pathogenic to humans (Table 1) (Jonsson et al. 2010). The genus Hantavirus can be divided into two groups: Old World and New World hantaviruses. Pathogenic Old World hantaviruses comprise Asian and European viruses and New World hantaviruses North and South America viruses. The main natural reservoirs of these organisms are muroid rodents (Table 1): the Old World rats and mice (family Muridae, subfamily Murinae), the New World rats and mice (family Cricetidae, subfamily Sigmodontinae) and the voles and lemmings (family Cricetidae, subfamily Arvicolinae). The most widely distributed rodent source of hantaviruses in Europe is the bank vole, the carrier of PUUV (Jonsson et al. 2010). This creature is found throughout Europe, with the exception of the Mediterranean region and northern parts of Finland, Sweden and Norway (Vapalahti et al. 2003).

It seems that hantaviruses have coevolved with their rodent hosts for millions of years (Plyusnin et al. 1996). Such a conception is supported by molecular evidence showing a high degree of similarity between the phylogenetic structure of the host rodent and that of the viruses (Plyusnin and Morzunov 2001). However, it is possible that hantavirus has multiple rodent hosts and multiple viruses have a single host species (Nemirov et al. 2002, Scharninghausen et al. 2004, Weidmann et al. 2005). The distribution of discrete hantavirus species appears to correlate with the geographic extension of their hosts, and the hantavirus genotypes of the same

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geographic area are phylogenetically related (Plyusnin and Morzunov 2001, Plyusnin 2002).

Table 1. Human pathogenic hantaviruses with recognized clinical disease and their geographic distribution.[1]

Group and subfamily	Virus	Rodent host	Disease	Area
Old World				
Murinae	Hantaan	*Apodemus agrarius*	HFRS	Asia
	Dobrava	*Apodemus flavicollis*	HFRS	Europe
	Seoul	*Rattus norvegicus*	HFRS	Worldwide
	Saaremaa	*Apodemus agrarius*	HFRS	Europe
	Amur	*Apodemus peninsulae*	HFRS	Asia
Arvicolinae	Puumala	*Myodes glareolus*	HFRS/NE	Europe
New World				
Sigmodontinae	Sin Nombre	*Peromyscus maniculatus*	HCPS	North America
	New York	*Peromyscus leucopus*	HCPS	North America
	Monongahela	*Peromyscus leucopus*	HCPS	North America
	Black Creek Canal	*Sigmodon hispidus*	HCPS	North America
	Bayou	*Oryzomys palustris*	HCPS	North America
	Choclo	*Oligoryzomys fulvescens*	HCPS	South America
	Andes	*Oligoryzomys longicaudatus*	HCPS	South America
	Bermejo	*Oligoryzomys chocoensis*	HCPS	South America
	Lechiguanas	*Oligoryzomys flavescens*	HCPS	South America
	Maciel	*Bolomys obscurus*	HCPS	South America
	Oran	*Oligoryzomus longicaudatus*	HCPS	South America
	Laguna Negra	*Calomys laucha*	HCPS	South America
	Araraquara	*Bolomys lasiurus*	HCPS	South America
	Juquitiba	*Oligoryzomus nigripes*	HCPS	South America

[1]Table adopted and modified from the article by Jonsson et al. 2010. Only hantaviruses known to induce a recognized clinical disease in humans are included.

Hantaviruses are enveloped ribonucleic acid (RNA) viruses with diameter varying from 70 to 120 nm (Figure 1) (Vaheri et al. 2011). They are composed of a spherical lipid envelope, four viral proteins and three single-stranded, negative-sensed RNA segments designated S (small), M (medium) and L (large) which code for the nucleocapsid protein (NP), the surface envelope glycoproteins Gn and Gc, and the RNA-dependent RNA polymerase, respectively (Figure 1). NP is the most abundant of these viral proteins, and also has an important role in the life cycle of the virus (Jonsson et al. 2010).

Figure 1. Hantavirus structure, genes and gene products. Hantaviruses have a tri-segmented (L, M, S) negative-stranded RNA genome. The termini of the segments are conserved and complementary to each other. Hantaviruses carried by *Cricetidae* (but not *Muridae*) rodents have in the S segment an additional open reading frame (ORF).
Reproduced with permission from Oxford University Press (Vaheri et al. 2011: Hantaviruses. In "Oxford Textbook of Zoonoses ─ Biology, Clinical Practice and Public Health Control" 2nd edition. Oxford University Press. Oxford U.K.)
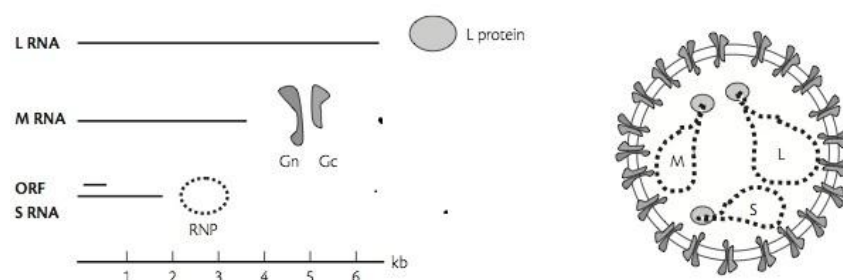
2.1.3 Ecology

Rodent carriers of hantaviruses are chronically and asymptomatically infected. They shed the virus into the environment in their urine, saliva and feces perhaps throughout their lifetime, shedding, however, probably being greatest during the first 2–8 weeks after infection (Lee et al. 1981, Yanagihara et al. 1985, Hutchinson et al. 1998, Hardestam et al. 2008). Recent data indicate that hantaviruses are particularly stable, and remain infectious for up to ten days at room temperature, in winter possibly longer (Kallio et al. 2006, Hardestam et al. 2007).

Humans do not belong to the natural host range of hantaviruses, and infection is suggested to occur via inhalation of virus-containing, aerosolized rodent excretions (Gajdusek 1982). In contrast, the Andes virus has been found to be transmittable from person to person (Padula et al. 1998), and also the transmission of PUUV via blood products has recently been suspected (Sinisalo et al. 2010). Nevertheless, living in close contact with infected rodents, work in professions such as forestry and farming, and outdoor activities in general are risk factors for HFRS (Ahlm et al. 1994a, Zöller et al. 1995, Vapalahti et al. 1999, Olsson et al. 2003). Typically, the risk of infection is increased in activities such as cleaning out barns or summer cottages, and inhaling contaminated dry dust in a poorly ventilated space (Zeitz et al. 1995, Crowcroft et al. 1999, Van Loock et al. 1999). Interestingly, recent data also show that cigarette smoking increases the risk of NE (Vapalahti et al. 2010). This supports the previous conception that hantavirus transmission occurs by inhalation, the condition of the respiratory tract possibly contributing to the infection.

In Northern Europe, bank voles undergo a 3–4 year cycle of population irruptions. These cycles are linked to the inter-annual variation in the incidence of

HFRS, and have been explained by variations in predator populations and availability of food (Crespin et al. 2002, Hörnfeldt 2004). During the peak phases of the bank vole population cycle, vole densities are high from the late summer of the peak year to late winter 1.5 years later (Vapalahti et al. 2003). The majority of NE cases occur from November to February, with a minor peak in PUUV infections also seen in August (Vapalahti et al. 2003, Heyman et al. 2009). The possible explanation for NE cases appearing during the late autumn and early winter is that the rodents are moving into human dwellings to avoid the cold winter (Niklasson and LeDuc 1987). Summer cases are probably a consequence of urban dwellers being infected during their summer vacations (Heyman et al. 2009). However, in continental Europe, the high epidemic years are predicted by the climate and tree seed production, conspicuous bank vole abundance and the highest numbers of NE cases occuring during the summer (Tersago et al. 2009).

2.1.4   Epidemiology

Worldwide, approximately 200 000 cases of HFRS are hospitalized annually (Muranyi et al. 2005). Globally most HFRS cases occur in the Far East, but the incidences of hantavirus infections are influenced in part by the use of diagnostic tests. In Europe, PUUV is the most common cause of HFRS. There is an increasing trend in the incidence of NE, with an average annual figure of 31/100 000 (Makary et al. 2010). In Finland 3200 cases of NE were recognized in 2008, but the incidence during recent years has usually ranged from 1000 to 3000 cases per year (National Institute of Health and Welfare, Finland 2010). In Sweden there are around 100–300 HFRS cases per year and in Norway around 50 cases (Vapalahti et al. 2003). In 2007, however, there were over 2000 PUUV infections in Sweden (Heyman et al.

2009). Climate change has probably contributed to the rise. In Northern Europe mild winters cause lack of snow cover and force rodents to seek shelter from the cold and predators in houses and other buildings (Pettersson et al. 2008a, Evander and Ahlm 2009). In continental Europe, a combination of high summer temperatures and low precipitation, and high tree seed production has led to increases in bank vole populations, and also an increased incidence of NE cases (Tersago et al. 2009).

The average PUUV seroprevalence in Finland is 5 % (in eastern Finland 11 %), suggesting that many infections remain subclinical and undiagnosed (Brummer-Korvenkontio et al. 1999). Furthermore, antibodies to PUUV are present in 5.4 % of the healthy population in northern Sweden (Ahlm et al. 1994a), 5.1 % in Estonia and 3.4 % in Saaremaa (Golovljova et al. 2002). Recent registry data on 22681 NE cases from Finland showed that 62 % of cases were males, and the highest incidences were observed in the age groups 34-49 years and 50-64 years (Makary et al. 2010). The reasons why children are less likely to suffer from HFRS, and why their infections seem to be milder than in adults remain obscure (Mustonen et al. 1994c, Huttunen et al. 2011).

In the Balkans PUUV is also probably the most frequent cause of HFRS (Lundkvist et al. 1997, Hukic et al. 2010). However, Dobrava virus (DOBV), isolated from the yellow-necked mouse (*Apodemus flavicollis*) in Slovenia in 1988 (Avsic-Zupanc et al. 1992), is the main cause of severe HFRS in the area (Antoniadis et al. 1996, Lundkvist et al. 1997, Avsic-Zupanc et al. 1999, Markotic et al. 2002). An epidemic of more than 2000 cases of DOBV infection was reported from 1995 to 1996 in Yugoslavia (Skataric et al. 1998) (cited in Bi et al. 2008), and the hantavirus antibody seroprevalences have been reported to be 5 % in Bosnia, 4 % in Greece, 1.7 % in Slovenia and 1-6 % in Croatia (Heyman et al. 2009).

Saaremaa virus (SAAV), antigenically closely related to DOBV, was detected in field mice (*Apodemus agrarius*) in Estonia in the 1990s (Plyusnin et al. 1997a, Nemirov et al. 1999, Avsic-Zupanc et al. 2000). However, compared to DOBV, SAAV probably causes a milder type of HFRS (Golovljova et al. 2007). Furthermore, the European common vole (*Microtus Arvalis*) is a carrier of Tula virus, which appears to be apathogenic to human, but has also been linked to a clinical infection (Plyusnin et al. 1994, Vapalahti et al. 1996a, Schultze et al. 2002).

In Russia there are a number of hyperendemic areas with high incidences of HFRS (58/100 000), although the figure is lower in the European part (5/100 000) (Vapalahti et al. 2003). Most cases are probably caused by PUUV (Vapalahti et al. 2003). China has been the most markedly endemic country for HFRS, with 40000–60000 cases reported annually during the last few years (Bi et al. 2008). Besides HTNV, in Asia Seoul virus, transmitted by urban rat (*Rattus norvegicus*), was shown to cause HFRS in Seoul in 1980s (Table 1) (Lee et al. 1982b). Furthermore, Amur virus has been isolated from the Korean field mouse (*Apodemus peninsulae*) (Yashina et al. 2001) causing HFRS in the area of Asia, and Thailand virus has been isolated from the bandicoot rat (*Bandicota indica*) (Pattamadilok et al. 2006, Nakamura et al. 2008).

The first pathogenic hantavirus in the New World was discovered in 1993 in the southwestern part of the United States, causing hantavirus pulmonary syndrome, currently named hantavirus cardiopulmonary syndrome (HCPS). It was detected in deer mice (*Peromyscus maniculatus*), and named Sin Nombre virus (SNV) (Nichol et al. 1993). Other hantaviruses causing human disease in North America are listed in Table 1. From 1993 to March 2007 465 HCPS cases were reported from the United States (Jonsson et al. 2010). The first cases of HCPS in South America were

recognized in Brazil in 1993, caused by Juquitiba virus (Vasconcelos et al. 1997), and several other pathogenic hantaviruses have since been detected (Table 1). Interestingly, as far back as in 1982 Prospect Hill virus, apathogenic to humans, had been isolated from a meadow vole (*Microtus Pennsylvanicus*) in the United States (Yanagihara et al. 1984).

2.2 Clinical features of hantavirus infections

2.2.1 Clinical, laboratory and radiological findings in nephropathia epidemica

The incubation period of acute PUUV infection has been estimated to vary from 1 to 8 weeks (Settergren et al. 1989). Usually, the condition is characterized by a sudden onset of fever and headache, nausea, backache and abdominal pains. The most typical symptoms and laboratory findings in acute NE are described in detail in 4 studies involving from 37 to 126 hospitalized patients and shown in Table 2 (symptoms) and Table 3 (laboratory findings) (Lähdevirta 1971, Settergren et al. 1989, Mustonen et al. 1994a, Braun et al. 2010). Typical renal findings are the phase of oliguria or anuria followed by polyuria (Table 2). Proteinuria, hematuria and increased serum creatinine levels are common (Table 3).

Although NE belongs to the group of hemorrhagic fevers, serious hemorrhagic complications are rare. Epistaxis is found in up to 28 % of patients (Settergren et al. 1989). Hemoptysis and diffuse bleedings from mucosal membranes have also been described (Settergren et al. 1988, Forslund et al. 1992). Hematemesis and melena are seen in a minority of the NE cases (Table 2), but hemorrhagic gastropathy is probably more usual (Nuutinen et al. 1992).

Table 2. Clinical symptoms and findings in patients with PUUV infection.
References: a=Lähdevirta 1971, b=Settergren et al. 1989, c=Mustonen et al. 1994a, d=Braun et al. 2010.

Symptom or finding	Percentages of symptoms or findings (%)	References
General symptoms		
Fever	98-100	a,b,c,d
Edema	9-59	a,b,c
Lymphadenopathy	2-15	a,b,c
Petechial rash	1-12	a,b,c
Pain		
Backache	33-82	a,b,c,d
Abdominal pains	30-67	a,b,c,d
Myalgia	27-69	b,c,d
Arthralgia	1-15	b,c
Neurologic		
Headache	34-90	a,b,c,d
Dizziness	9-34	a,b,c
Meningism	1-5	a,c
Nephrologic		
Tenderness in kidney area	48-84	a,c
Oliguria/anuria	34-70	a,b,c,d
Polyuria	45-100	a,b,c,d
Visible hematuria	1-7	a,b,c
Dysuria	<1-12	b,c,d
Ophthalmologic		
Visual disturbance	31-36	b,c
Sensitivity to light	7-10	b,c
Conjunctival bleeding	6-9	a,c
Gastroenterologic		
Nausea	58-84	a,b,c
Vomiting	51-70	a,b,c
Diarrhea	7-20	a,b,c,d
Constipation	8-34	a,b,c
Melena/hematemesis	1-2/1	b,c
Respiratory		
Red throat/tonsil exudates	19-67	a,c
Cough	14-32	b,c

Table 3. Laboratory findings in patients with acute PUUV infection.

Finding	Percentages of abnormal findings (%)			
	Lähdevirta[1] (n=16-76)	Settergren[2] (n=74)	Mustonen[3] (n=126)	Braun[4] (n=75)
Leukocytosis	57	NA	50	36
Thrombocytopenia	57	NA	75	69
Elevated plasma CRP	NA	96	NA	100
Decreased serum albumin	88	NA	50	NA
Elevated serum creatinine	85	96	NA	96
Proteinuria	100	100	94	82
Microscopic hematuria	74	85	58	65

References: [1]Lähdevirta 1971, [2]Settergren et al. 1989, [3]Mustonen et al. 1994a, [4]Braun et al. 2010. Abbreviations: CRP=C-reactive protein, NA=not available.

Thrombocytopenia is common (Table 3), and anemia is seen in 50 % of cases (Mustonen et al. 1994a). Recently, thrombocytopenia was found to be associated with a decrease in natural anticoagulants, shortened thrombin time and enhanced fibrinolysis (Laine et al. 2010) as well as activation of the thrombocytes (Laine et al. 2011). Disseminated intravascular coagulation was also diagnosed in 26 % of NE patients (Laine et al. 2010). The possible mechanisms underlying anemia are infection as such and acute renal failure, perhaps also aggravated by a bleeding tendency. However, high blood haemoglobin values may also be observed in the

early phase of NE, suggested to be a sign of hemoconcentration caused by increased capillary permeability (Kanerva et al. 1998a). Another possible mechanism involved in hemoconcentration is dehydration due to fever and vomiting (Lähdevirta 1971).

Leukocytosis in NE is usually mild (Lähdevirta 1971, Mustonen et al. 1994a, Braun et al. 2010), and shifts to the left in differential counts of leukocytes (Lähdevirta 1971) and the presence of atypical lymphocytes can be detected as well (Settergren et al. 1989, Braun et al. 2010). A case with pronounced monocytosis has been described as well (Ala-Houhala et al. 2000). Increased levels of plasma C-reactive protein (CRP) are found in almost all cases (Table 3). It has recently been shown, however, that high plasma CRP does not reflect the severity of the disease, but might even have a protective effect on renal function during acute NE (Outinen et al. 2010).

Further, elevated levels of the liver enzymes are usual (Lähdevirta 1971, Settergren et al. 1989), although in a recent study from Germany they remained essentially unaffected (Braun et al. 2010). Transient electrolyte abnormalities such as hypocalcemia, hyponatremia, hyperphosphatemia, hypokalemia and hyperkalemia are often recorded (Mustonen et al. 1994a), and they are mainly related to acute renal failure. Decreased serum albumin levels have also been reported in most cases (Table 3), mainly associated with acute infection and often heavy proteinuria.

Ocular symptoms and visual disturbances are commonly encountered during acute NE (Table 2), and the incidence of transient myopia has been found to be 8 % (Kontkanen et al. 1994). In a recent Finnish study, a total of 70 % of NE patients complained of ocular symptoms, and myopic refractation change was found in 74 % of cases (Hautala et al. 2010). Diminution of visual acuity affected 87 % and

decreased intraocular pressure 88 % of patients (Hautala et al. 2010). Since these visual disturbances were only partially explained by the myopic shift, there may also be extraocular mechanisms in the etiology of ocular symptoms. Further, conjunctival bleedings (Lähdevirta 1971, Mustonen et al. 1994a) and chemosis (Hautala et al. 2010) have been described during acute NE.

Abnormal electrocardiogram (ECG) findings were observed in one third of 70 PUUV-infected patients in a study from Croatia (Puljiz et al. 2005). In a Finnish study of 70 NE patients, 57 % presented with ECG changes (Mäkelä et al. 2009). Transient T-wave inversions have proved to be the most common findings (Mäkelä et al. 2009), and sinus tachycardia and bradycardia, and conduction defects have also been detected (Puljiz et al. 2005). In a study by Mäkelä and co-workers (2009), 16 % of patients showed abnormalities in echocardiography (ECHO). Impaired contraction of the left ventricle was the most common finding, and one patient also had pericardial fluid. The abnormal ECHO findings could suggest myocarditis, although plasma troponin I levels were normal in all patients (Mäkelä et al. 2009). These results could also indicate myocardial dysfunction caused by fluid retention, abnormal plasma electrolyte levels, fever and cytokine release during acute infection. Nevertheless, during the follow-up both ECG and ECHO abnormalities reverted to normal in all patients, which would indicate their benign nature. However, acute clinical perimyocarditis may complicate acute NE (Lähdevirta 1971, Mustonen et al. 1994a), and has also been reported in fatal cases (Valtonen et al. 1995).

Abnormal findings in chest radiography are reported in 16-35 % of PUUV-infected patients (Lähdevirta 1971, Mustonen et al. 1994a, Kanerva et al. 1996, Paakkala et al. 2004). The most usual findings are pleural effusions, atelectasis and

interstitial infiltrates (Lähdevirta 1971, Mustonen et al. 1994a, Kanerva et al. 1996, Paakkala et al. 2004). Frank pulmonary edema is rare (Kanerva et al. 1996), but in a few NE cases the clinical picture of the disease has been reported to be similar to HCPS with acute non-cardiogenic pulmonary edema (Clement et al. 1994, Launay et al. 2003, Seitsonen et al. 2006, Rasmuson et al. 2011a).

Data on the clinical picture of NE in children are limited. However, the symptoms and clinical findings largely resemble those in adults, although the disease is usually milder in children than in adults (Mustonen et al. 1994c, Huttunen et al. 2011). Nausea, vomiting and abdominal pain have been reported more often in children than in adults (Ahlm et al. 1994b, Mustonen et al. 1994c). A recent systematic literature review likewise confirmed the clinical impression that NE is milder in children (Huttunen et al. 2011). Severe complications and deaths have been reported only in adult patients.

2.2.2 Renal disease in nephropathia epidemica

Back pain and tenderness of the kidneys are common symptoms in acute NE (Table 2). Polyuria following the oliguric phase is reported in 45-100 % of patients, and may result in daily urine volumes up to 10 litres (Lähdevirta 1971, Settergren et al. 1989, Mustonen et al. 1994a, Mäkelä et al. 2004, Braun et al. 2010). However, polyuria may also been seen without preceding oliguria indicating tubular dysfunction (Lähdevirta 1971).

Proteinuria is the most common urinary sediment abnormality in patients with acute NE, detected in up to 100 % of cases (Lähdevirta 1971, Settergren et al. 1989, Mustonen et al. 1994a, Braun et al. 2010). It appears on the third to fifth day from the onset of fever, and peaks on average on day eight (Kanerva et al. 1998a).

Nephrotic range proteinuria (> 3.5 g/day) is observed in 25-34 % of patients (Mustonen et al. 1994a, Mäkelä et al. 2004). Proteinuria in acute HFRS is non-selective (Cosgriff 1991). Besides albumin, low-molecular-weight proteins such as β2-microglobulin, α1-microglobulin and immunoglobulin G (IgG) are detected in the urine during the acute phase of NE, contributing the proteinuria and reflecting tubular dysfunction (Settergren et al. 1990, Cosgriff 1991, Ala-Houhala et al. 2002, Mäkelä et al. 2004). Increased glomerular permeability is associated with both the size- and charge-selectivity properties of the glomerular filter in acute NE patients (Ala-Houhala et al. 2002). The pathogenesis of proteinuria in acute NE is not known. Immunological mechanisms may be important, as suggested by a positive correlation between increased urinary excretion of interleukin 6 (IL-6) and proteinuria (Mäkelä et al. 2004). However, proteinuria rapidly disappears during the polyuric phase of acute NE.

Microscopic hematuria is detected in 58-85 % of cases with acute NE (Lähdevirta 1971, Settergren et al. 1989, Mustonen et al. 1994a, Braun et al. 2010), but macroscopic hematuria is rare (Table 2). Other urinary sediment findings reported are leukocyturia, granular and hyaline casts, and glucosuria (Lähdevirta 1971, Settergren et al. 1989, Mustonen et al. 1994a, Braun et al. 2010).

Transient impairment of renal function affects most hospitalized NE patients (Lähdevirta 1971, Settergren et al. 1989, Mustonen et al. 1994a, Braun et al. 2010). In the study by Settergren and co-workers (1989), 35 % of patients had serum creatinine levels over 500 μmol/l, the median value being 386 μmol/l. In one Finnish study the mean serum creatinine value was 439 μmol/l (Mustonen et al. 1994a). Measurement of the glomerular filtration rate (GFR) by ^{51}Cr-EDTA or inulin clearance yields markedly reduced results during the acute phase of NE (Settergren

et al. 1990, Ala-Houhala et al. 2002). Ala-Houhala and co-workers (2002) have also shown that the effective renal plasma flow (ERPF) is significantly reduced and the filtration fraction (FF) markedly increased. Hypovolemia may contribute to these functional changes. However, acute renal failure may also affect patients without hypotension, which would point to intrarenal mechanisms of kidney injury (Cosgriff 1991). The need for dialysis treatment is reported in 0-6 % of hospitalized NE cases (Settergren et al. 1989, Mustonen et al. 1994a, Mäkelä et al. 2004, Braun et al. 2010, Hukic et al. 2010), distinctly fewer compared with 16 % (Hukic et al. 2010) or even 47 % in patients with DOBV infections (Avsic-Zupanc et al. 1999).

Renal biopsies performed during the acute phase of NE reveal acute tubulointerstitial nephritis with minor glomerular changes (Collan et al. 1991, Mustonen et al. 1994b). The most characteristics lesions are interstitial edema and diffuse or focal inflammatory cell infiltrations consisting mainly of lymphocytes, monocytes, macrophages, plasma cells and eosinophilic granulocytes (Collan et al. 1991, Mustonen et al. 1994b, Temonen et al. 1996). Further, congestion or hemorrhages of the vessels in the outer medulla or corticomedullary junction have been detected (Collan et al. 1991, Mustonen et al. 1994b). Especially the interstitial hemorrhages should alert the pathologist to the possibility of NE (Mustonen et al. 1994b). Tubular lumen dilatation and epithelial flattening as well as tubular casts are also common findings (Collan et al. 1991, Mustonen et al. 1994b). Mustonen and co-workers (1994b) found slight glomerular mesangial alterations, mainly hypersellularity or sclerosis, in 25 % of cases. Interestingly, the amount of proteinuria had no relation to the severity of histological findings in renal biopsy, but the highest serum creatinine level showed a slight correlation with tubular and interstitial findings (Mustonen et al. 1994b). Compared with the amount of

albuminuria seen during acute NE, the glomerular changes are surprisingly mild. In fatal cases of PUUV (Forslund et al. 1992, Valtonen et al. 1995) and DOBV infections (Avsic-Zupanc et al. 1999), hemorrhages and occasionally necrosis of the kidneys have been reported.

Immunofluorescence (IF) studies show no specific deposits in renal biopsies performed during acute NE (van Ypersele de Strihou and Mery 1989, Mustonen et al. 1994b). Van Ypersele de Strihou and Mery (1989) reported mainly negative IF findings, but on a third of the biopsies complement (C) component 3 could be detected in the arterial walls. In one of the aforementioned Finnish studies, glomerular IF was negative in 43 % of biopsies (Mustonen et al. 1994b). Mainly mild mesangial and granular IgM, IgG, IgA, C3 or C1q were found. In one case a diagnosis of IgA nephropathy (IgAN) could be reached. Tubular and arteriolar C3 was detected in a minority of the cases. Collan and co-workers (1978a, 1978b) studied 20 renal biopsy specimens from clinically diagnosed NE patients using electron microscopy. They found collections of light flocculent material under the endothelial cells and various deposits in the mesangium as well as splitting and thickening of the cortical and medullary tubular basement membranes.

In the acute phase of NE, renal ultrasound study reveals an abnormal resistive index of renal parenchyma in half of the patients, and this is positively associated with the degree of clinical renal failure (Paakkala et al. 2002). Increased cortical echoicity and swelling as well as disturbances in corticomedullary border differentiation and perirenal fluid collections may also be seen, and altogether 47 % of NE patients may yield abnormal findings in renal ultrasound (Paakkala et al. 2004). In HFRS patients, low signal intensity has been shown especially in the outer renal medulla by magnetic resonance imaging (MRI) probably representing

medullary hemorrhage (Kim et al. 1990). More recently, Paakkala and co-workers (2005) showed that renal parenchymal volume, renal length and parenchymal thickness are increased in MRI during acute NE, and these changes show a mild association with disease severity. Paakkala and co-workers (2006) have also performed magnetic resonance renography in acute NE patients and detected an abnormal uptake slope of the contrast enhancement curve. This finding likewise showed a mild positive correlation with the clinical severity of the renal insufficiency and fluid volume overload.

2.2.3 Central nervous system symptoms and findings in nephropathia epidemica

Central nervous system (CNS) symptoms are usual during acute NE (Lähdevirta 1971, Settergren et al. 1989, Mustonen et al. 1994a, Alexeyev and Morozov 1995, Ahlm et al. 1998, Braun et al. 2010, Hautala et al. 2010). The most common CNS symptom is headache, which affects up to 97 % of patients (Alexeyev and Morozov 1995). Other typical manifestations include insomnia in 83 % and vertigo in 79 % of patients (Ahlm et al. 1998), somnolence in 63 % and restlessness in 25 % (Lähdevirta 1971), and anxiety in 18 % (Settergren et al. 1989). Nausea and vomiting also characterize acute NE (Table 2), and may reflect the involvement of the CNS (Ahlm et al. 1998).

Intracerebral hemorrhages predominantly in the region of the pituitary gland concomitant with necrosis have been diagnosed radiologically or found in autopsy studies in some cases with NE (Valtonen et al. 1995, Hautala et al. 2002, Hautala et al. 2010). In brain MRI, unspecific pituitary abnormalities such as uneven distribution of the contrast media and suspected edema have also been described

(Hautala et al. 2010). Furthermore, there are few reports of panhypopituitarism diagnosed during the acute phase of HFRS (Park and Pyo 1996, Hautala et al. 2002, Hautala et al. 2010), in one case only temporary (Hautala et al. 2010). Interestingly, PUUV antigen has been detected in the hypophysis in an autopsy of an NE patient (Hautala et al. 2002).

One recent prospective study of 58 NE patients showed that altogether 87 % of patients experienced symptoms consistent with CNS involvement (Hautala et al. 2010). Severe manifestations are, however, rarely reported. In a review of 811 NE cases, the incidence of severe neurological symptoms such as meningism, cerebral hemorrhage or seizures was only 1 % (Alexeyev and Morozov 1995). As rare phenomena, again, encephalitis (Launes and Hautanen 1988, Mustonen et al. 1994a, Bergmann et al. 2002, Hautala et al. 2002, Hautala et al. 2010), acute disseminated encephalomyelitis (Toivanen et al. 2002, Krause et al. 2003), meningism or seizures (Alexeyev and Morozov 1995), Guillain-Barre syndrome (Forslund et al. 1992, Esselink et al. 1994), and urinary bladder paralysis (Alexeyev and Morozov 1995) have been reported to complicate acute NE.

In NE patients with CNS symptoms, analysis of cerebrospinal fluid specimens has revealed pleocytosis (Hautala et al. 2002, Hautala et al. 2010) or increased protein levels (Lähdevirta 1971, Settergren et al. 1988, Settergren et al. 1989, Forslund et al. 1992, Ahlm et al. 1998, Hautala et al. 2002, Hautala et al. 2010). Recently, PUUV RNA was also successfully detected in the cerebrospinal fluid of an NE patient evincing neurological symptoms (Mähönen et al. 2007, Hautala et al. 2010). In one Swedish study, brain MRI showed minor, unspecific white matter lesions in about half of the patients investigated (Ahlm et al. 1998). In patients with encephalitis or acute disseminated encephalomyelitis, brain MRI has also revealed

abnormal signal intensity (Bergmann et al. 2002, Toivanen et al. 2002, Krause et al. 2003). Furthermore, in one aforementioned Swedish study of 20 NE patients, electroencephalography (EEG) showed severe changes in one patient with seizures (Ahlm et al. 1998), while in a more recent study by Hautala and co-workers (2010), the EEGs of 33 NE patients were within normal limits.

2.2.4 Clinical picture of nephropathia epidemica compared with other hemorrhagic fever of renal syndromes

DOBV as well as HTNV infections usually cause similar, but more severe clinical courses of HFRS than PUUV infection (Lee and van der Groen 1989, Avsic-Zupanc et al. 1999). The five phases of HFRS (febrile, hypotensive, oliguric, diuretic and convalescent) described in HTNV-induced HFRS (Lee and van der Groen 1989) are not always clinically evident in acute NE. Mild and moderate cases constitute about 90 % of all NE cases (Lähdevirta 1971). In the Balkans, the clinical picture of DOBV infection appears to be more severe than that of PUUV infection (Avsic-Zupanc et al. 1999, Markotic et al. 2002, Hukic et al. 2010, Tulumovic et al. 2010). For example, severe hemorrhagic complications and shock have been described in DOBV but not in PUUV infections (Avsic-Zupanc et al. 1999). The reasons for the different clinical courses of hantavirus infections are unknown, but possibly the virulence of the individual virus, the infective dose or host factors contribute to the severity of the disease (Kanerva et al. 1998a).

Mortality rates in PUUV infection range from 0.08 % in Scandinavia (Makary et al. 2010) to 0.4 % in Russia (Vapalahti et al. 2003). Only sporadic fatal cases have been described in Scandinavia (Linderholm et al. 1991, Forslund et al. 1992, Valtonen et al. 1995, Hautala et al. 2002). Lethality in PUUV infections has proved

attributable to shock and hemorrhages in several organs. In contrast, the mortality rate in DOBV infections has been reported to be 16 % (Avsic-Zupanc et al. 1999), while in HTNV infections it varies from 5 to 15 % (Lee 1996) (cited in Schmaljohn and Hjelle 1997).

2.2.5 Hantavirus cardiopulmonary syndrome

HCPS is a severe form of hantavirus infection typically characterized by severe pulmonary involvement. It comprises four clinical phases: prodrome, pulmonary edema and shock, diuresis and convalescence (Simpson et al. 2010). Cough is present in 60 % of HCPS patients on presentation, but dyspnea is not common until the phase of respiratory failure (Simpson et al. 2010). Ten percent of patients may suffer from severe abdominal tenderness during the prodromal phase. Thrombocytopenia develops in all patients, while petechiae are rare (Simpson et al. 2010). The renal involvement in HCPS is not as marked as in HFRS, although proteinuria is detected in 40 % and hematuria in 57 % of HCPS cases on admission (Duchin et al. 1994). Serum creatinine values higher than 177 µmol/l have been detected in 20 % of HCPS patients (Peters et al. 1999). In case series from South America, the incidence of elevated serum creatinine values varied from 48 % to 54 %, whereas severe renal failure and the need for dialysis treatment were uncommon (Castillo et al. 2001, Riquelme et al. 2003). Interestingly, the clinical picture of acute NE may in rare cases also resemble that of HCPS with noncardiogenic pulmonary edema (Clement et al. 1994, Launay et al. 2003, Seitsonen et al. 2006, Rasmuson et al. 2011a). These findings suggest the possibility of over-lapping in the clinical pictures of HFRS and HCPS.

Hypotension developing in the shock phase requires rapid, aggressive resuscitation (Simpson et al. 2010). Rapidly progressive pulmonary edema and hemorrhage may lead to respiratory distress syndrome requiring mechanical ventilation (Hallin et al. 1996, Dietl et al. 2008). Myocardial dysfunction is usual in the shock phase (Hallin et al. 1996), and recently Saggioro and co-workers (2007) showed typical myocarditis with hantaviral particles within endothelial and macrophage-type cells in an autopsy study. The authors thus proposed the name hantavirus cardiopulmonary syndrome instead of hantavirus pulmonary syndrome.

Mortality in HCPS is very high. In data on 465 reported HCPS cases in the United States, the percentage of cases resulting in death was 35 % (Jonsson et al. 2010). In case series mortality rates have been even higher, ranging from 59 % (Nolte et al. 1995) to 76 % (Duchin et al. 1994), and are highest during the first 24 hours of shock phase. A detailed description of clinicopathological findings in 44 fatal HCPS cases revealed multiorgan involvement with vascular congestion, but the main pathological findings were substantial pleural effusions and interstitial pneumonitis with congestion, edema and mononuclear cell infiltration (Zaki et al. 1995). The alveoli contained abundant edema fluid and fibrin together with inflammatory cells as well as focal hyaline membranes. In the kidneys, no significant glomerular or tubulointerstitial changes were detected, although SNV antigens were found in the medulla and glomeruli.

2.2.6   Diagnosis, treatment and prevention

Acute hantavirus infection is currently diagnosed by an IgM enzyme immunoassay (EIA) test based on recombinant full-length hantavirus nucleocapsid protein (Vaheri et al. 2008). Rapid immunochromatographic IgM tests are also available for several

hantaviruses (Hujakka et al. 2001, Hujakka et al. 2003), and IgG EIAs can be used to confirm previous hantavirus infection. Hantaviral RNA can also be found in peripheral blood mononuclear cells, serum samples, urine, saliva or cerebrospinal fluid, but polymerase chain reaction (PCR) methods are not in routine use (Plyusnin et al. 1997b, Plyusnin et al. 1999, Evander et al. 2007, Mähönen et al. 2007, Pettersson et al. 2008b).

Currently, no specific therapy for HFRS or HCPS is available. Of prime importantance is careful monitoring and supportive treatment of fluid balance, diuresis and respiration as well as pain relief (Vapalahti et al. 2003). Recently, Dietl and co-workers (2008) showed that in HCPS extracorporeal membrane oxygenation may reduce the complication rate and improve overall survival. HFRS patients may need hemodialysis sessions. In a prospective, randomized, double-blind study of antiviral agent ribavirin therapy in HFRS patients in China a sevenfold reduction in mortality was achieved compared with placebo (Huggins et al. 1991). Likewise, the risk of severe renal failure diminished almost fourfold, this probably contributing to the reduction in mortality. However, the total numbers of deaths in that study were small. A recent study of HNTV patients in Korea also suggested a decrease in renal complications in HFRS patients treated with ribavirin (Rusnak et al. 2009). In contrast, one randomized study treating HCPS patients with ribavirin showed no benefit of the treatment (Mertz et al. 2004). Ribavirin has not so far been studied in Europe in the context of PUUV or DOBV infections. Two case reports have been published on the possible benefit of corticosteroids in PUUV infection. One involved treatment of marked and prolonged thrombocytopenia (Dunst et al. 1998), the other severe NE resembling HCPS (Seitsonen et al. 2006). Nevertheless, the role of corticosteroids in acute NE remains unclear.

Vaccines for HFRS have shown promise in clinical studies in Asia, but for European HFRS or HCPS there are for the present no available vaccines (Schmaljohn 2009). The only effective means of preventing hantavirus infection is avoidance of contact with rodents and rodent-occupied environments. It has been estimated that most HCPS cases (Armstrong et al. 1995) and Northern European HFRS cases (Olsson et al. 2003) occur in peridomestic areas. Holes in human dwellings should be blocked to prevent the entry of rodents (Vapalahti et al. 2010). Unoccupied buildings should also be allowed to air out properly before entering and starting cleaning, and dead or captured rodents or their excreta should be removed wearing rubber gloves and mask. Contaminated surfaces should be made wet, and handled with disinfectant. Poisoning may be better than trapping for rodent control, since poisoned rodents usually leave human dwellings before dying, whereas trapped rodents need to be removed (Vapalahti et al. 2010). Persons in occupations handling rodents or having repeated contacts with them should be educated to protect themselves from infection.

2.3   Pathophysiology of hantavirus infections

2.3.1   Endothelial cell

It has not yet been determined how hantaviruses disseminate in the human body after inhalation, but immature dendritic cells may have an important role in that they may serve as vehicles for the virions to infect other immune cells, and they also express β3 integrins (Schönrich et al. 2008). Beta integrins are receptors for hantaviruses, allowing entry to the host cell, and β3 integrins are receptors for pathogenic and β1 integrins for apathogenic hantaviruses (Gavrilovskaya et al.

1998, Gavrilovskaya et al. 1999, Larson et al. 2005). Recently, a cell surface protein of the complement regulatory system, named decay-accelerating factor (DAF)/CD55, was also suggested to be important factor in hantavirus infection (Krautkrämer and Zeier 2008). Hantaviruses replicate in the cytoplasm, and the glycoproteins are targeted to the Golgi apparatus, where most hantaviruses bud (Spiropoulou 2001).

Hantaviruses have been shown to infect endothelial, epithelial, macrophage, follicular dendritic and lymphocyte cells (Zaki et al. 1995, Mackow and Gavrilovskaya 2001, Raftery et al. 2002, Markotic et al. 2007). However, both clinical and pathological findings demonstrate that pathogenic hantaviruses specifically target the endothelial cells, and hantaviral antigens have been found especially in the kidneys and the lungs of infected patients (Yanagihara and Silverman 1990, Nolte et al. 1995, Zaki et al. 1995, Kanerva et al. 1998a, Maes et al. 2004).

Increased capillary permeability and vascular leakage are the characteristic phenomena in hantavirus infection (Cosgriff 1991). However, the exact mechanisms involved in the pathogenesis of HFRS and HCPS are poorly understood. Beta3 integrins regulate vascular integrity, endothelial cell permeability and platelet aggregation (Mackow and Gavrilovskaya 2009), and hantaviruses may inhibit these regular β3 integrin functions, probably interfering with the endothelial permeability function. The role of β3 integrins in patients with hantavirus infection has not however been evaluated (Mackow and Gavrilovskaya 2009). There are no data regarding direct viral cytotoxicity *in vivo*, and hantavirus infection alone does not affect the permeability of endothelial cells *in vitro* (Yanagihara and Silverman 1990, Pensiero et al. 1992, Temonen et al. 1993, Nolte et al. 1995, Zaki et al. 1995).

Prolonged response to cytokines (Niikura et al. 2004) and complement activation (Sane et al. 2011) may contribute to the increased vascular leakage. Recently, it was also suggested that pathogenic hantaviruses causing HFRS and HCPS sensitize human endothelial cells to the permeabilizing effects of vascular endothelial growth factor (Gavrilovskaya et al. 2008). Altogether, it is assumed that antiviral processes in the host cells and immune mechanisms are the main factors underlying the development of vascular dysfunction (Cosgriff 1991, Maes et al. 2004).

2.3.2 Animal models

The use of rodent-based models is limited in hantavirus infections, as these viruses causes an asymptomatic and persistent infection in their natural hosts. However, the nonhuman primate Cynomolgus macaque (*Macaca fascicularis*) can be experimentally infected with wild-type PUUV directly from *Myodes glareolus*, and develops symptoms and signs resembling human NE (Klingström et al. 2002). PUUV infection in the macaque resulted in lethargy, anorexia, proteinuria and hematuria, in addition to cytokine responses of interleukin 6 (IL-6), IL-10 and tumor necrosis factor alpha (TNF-α), as well as CRP, creatinine and nitric oxide (NO) responses. The increased titres of TNF-α seemed to correlate with a severe course of infection. The clinical picture of the disease also differed between the macaques as in humans. Recently, viral RNA and NP were observed in the kidneys, spleen and liver tissues of macaques with PUUV infection (Sironen et al. 2008). Inflammatory cell infiltrations in the kidneys contained mainly CD8-type T-cells, and they co-localized with the markers of viral replication in the kidneys at the sites of tissue damage.

2.3.3   Immune mechanisms

2.3.3.1   Cellular and humoral immunology and immunocomplexes

Cytotoxic T lymphocytes (CTLs) are assumed to have an important role in the pathogenesis of hantavirus infections (Maes et al. 2004). Increased amounts of CD8^{+} cells have been observed at the onset of HFRS (Huang et al. 1994, Markotic et al. 1999) and HCPS (Ennis et al. 1997, Kilpatrick et al. 2004), and have also been found in the lungs of patients dying from HCPS (Zaki et al. 1995). Higher numbers of CD8^{+} T cells have also been shown in bronchoalveolar lavage fluid (Linderholm et al. 1993) and in endobronchial mucosal biopsies (Rasmuson et al. 2011b) in patients with acute NE compared with healthy individuals. In patients with severe HCPS compared with those with moderate disease, the frequencies of SNV-specific CD8^{+} T cells in blood specimens have been particularly high (Kilpatrick et al. 2004). Huang and co-workers (1994) also showed reduced ratios of T helper cells (CD4^{+}) and CD8^{+} cells. In a recent study of HFRS patients, the peripheral subset of CD4^{+} cells appeared in reduced numbers compared with healthy individuals, and the frequency of the cells correlated positively with platelet count, and negatively with blood urea nitrogen, serum creatinine and serum aspartate aminotransferase (Zhu et al. 2009). In addition, a significant decrease in blood natural killer cells and an increase in these cells in bronchoalveolar lavage fluid have been found during the acute phase of NE, suggesting that natural killer cells migrate into infected tissue (Linderholm et al. 1993). In patients with NE, CD8^{+} memory cells have been shown to be rare during the acute phase, but gradually emerge in the convalescent phase (Tuuminen et al. 2007). It has also been shown that the virus-specific memory T cells persist for years after acute HTNV infection (Van Epps et al. 1999), Andes

hantavirus infection (Manigold et al. 2010), and PUUV infection (Van Epps et al. 2002).

In recent years, indirect evidence has accumulated supporting the role of CTLs in the dysfunction of endothelial cells and increased capillary permeability in hantavirus infection (Terajima et al. 2007). Hayasaka and co-workers (2007) have shown that specific CTLs increase the permeability of an immortalized human leukocyte antigen (HLA)-matched human endothelial cell monolayer infected with SNV. In addition, a recent study of PUUV infection has suggested activation of CD8$^+$ T cells and epithelial cell apoptosis (Klingström et al. 2006).

Interestingly, one very recent study has shown the high activity of indoleamine 2,3-dioxygenase (IDO) to be associated with higher maximum serum creatinine levels in acute NE patients (Outinen et al. 2011). IDO is an enzyme of amino acid metabolism expressed in immune cells such as macrophages and dendritic cells, and its increased activity probably leads to the inhibition of T-cell responses and proliferation (Outinen et al. 2011).

The nucleocapsid protein of hantavirus is the major target of the early antibody response in HFRS and HCPS, whereas neutralizing antibodies for Gn and Gc glycoproteins appear later (Lundkvist et al. 1993a, Groen et al. 1994, Vapalahti et al. 1995, Bostik et al. 2000, Maes et al. 2004). There is also evidence from animal models indicating that NP induces efficient protective immunity (Lundkvist et al. 1996, Maes et al. 2008). An IgM response is seen during the acute phase of PUUV infection, and IgG antibodies also appear during acute disease, but an increase in IgG-antibody titres is seen later (Lundkvist et al. 1993a, Groen et al. 1994). Furthermore, increased titres of total and virus-specific IgE have been detected during the acute phase of PUUV infection (Alexeyev et al. 1994), and elevated

levels of specific IgA during the acute phase of HFRS (Groen et al. 1994, de Carvalho Nicacio et al. 2000) and HCPS (Bostik et al. 2000). Relatively high titres of neutralizing antibodies have been detected many years after PUUV infection (Lundkvist et al. 1993b) and HCPS (Ye et al. 2004, Valdivieso et al. 2006). In addition, in HCPS patients the clinical course of the disease is more benign if the titres of neutralizing antibodies are high in the acute phase (Bharadwaj et al. 2000).

Activation of both the classical and alternative pathways of complement has been reported in acute NE, and shown to be associated with the severity of the disease (Paakkala et al. 2000, Sane et al. 2011). Immune complexes have also been demonstrated in serum, urine, glomeruli, tubules, skin and red blood cells as well as in platelets in HFRS patients (Jokinen et al. 1978, Penttinen et al. 1981, Chen and Yang 1990, Cosgriff 1991).

2.3.3.2   *Plasma and urinary cytokines and nitric oxide*

Several chemokines and cytokines are secreted in response to hantavirus infection (Muranyi et al. 2005). A study from Sweden showed in acute NE plasma TNF-α, IL-6 and IL-10 concentrations to be increased (Linderholm et al. 1996a). The maximum levels of plasma TNF-α concentration were seen 3 to 5 days after the onset of the infection. In a Finnish study, the serum concentrations of soluble IL-2 receptor (sIL-2R), IL-6 and IL-8 were higher in patients with acute NE compared with healthy controls (Takala et al. 2000). Further, Stoltz and co-workers (2007) reported significantly decreased interferon lambda (IFN-λ) serum levels in NE patients in the acute phase compared to those in the convalescent phase, whereas the serum levels of IFN-α and IFN-β did not differ between acute and convalescent phases. Interestingly, in one recent study the plasma levels of IL-9, fibroblast

growth factor 2, and granulocyte-macrophage colony-stimulating factor were shown to be higher in female patients compared with males during the acute phase of NE (Klingström et al. 2008). In the same study, the plasma levels of IL-8 and IFN-γ-induced protein 10 were lower in females than males. The authors concluded that the results may in part explain the higher rate of diagnosed NE in males than in females. However, the differences in plasma levels of cytokines no longer prevailed in the convalescent phase of the disease, implying that especially the acute cytokine responses are different between males and females (Klingström et al. 2008).

In the context of HTNV infection, Krakauer and co-workers (1994) detected elevated plasma levels of IFN-γ and IFN-α in 110 patients from the Korean War. Furthermore, in the same material IL-1β was more often detected in patients than in controls, while the percentage of detectable TNF-α did not differ between the groups (Krakauer et al. 1995). In HCPS, there are only limited data regarding the serum levels of different cytokines. Borges and co-workers (2008) showed higher serum IL-6 and TNF-α levels in HCPS patients than in healthy controls, but the levels of anti-inflammatory cytokine transforming growth factor-β were significantly decreased in patients compared with the control group.

Limited data have so far been published on the correlation of the clinical severity of human hantavirus infection with the serum levels of cytokines. A Finnish study of 19 patients with acute NE showed that serum levels of sIL-2R, IL-6 and IL-8 did not correlate with serum creatinine levels (Takala et al. 2000). However, sIL-2R correlated inversely with mean arterial pressure and minimum platelet count, and IL-6 also correlated inversely with minimum platelet count. In a Swedish study of 15 NE patients, maximum levels of TNF-α and IL-6 correlated positively with the maximum levels of serum creatinine, and TNF-α also correlated inversely with

mean blood pressure (Linderholm et al. 1996a). Further, in a Belgian study, the highest serum levels of creatinine were detected in NE patients with lowest TNF-α production *ex vivo* (Maes et al. 2006). Another recent Finnish study showed that NE patients with high plasma IL-6 levels had higher maximum blood leukocyte counts and urinary protein excretion as well as lower minimum platelet count, hematocrit and urinary output compared to patients with low plasma IL-6 levels (Outinen et al. 2010). Hospitalization was also longer in cases with high IL-6 levels. Furthermore, in HCPS, very high serum IL-6 levels are associated with hypotension and fatal outcome (Borges et al. 2008).

Recently, high frequencies of HTNV-specific IFN-γ-producing T cells were detected during the acute phase of infection, and reported to be higher in patients with maximum serum creatinine ≤ 707 μmol/l compared with those with more severe renal failure (maximum serum creatinine > 707 μmol/l) (Wang M et al. 2009). Moreover, the decrease in serum creatinine level was frequently accompanied by an increase in the magnitude of IFN-γ-producing T cells.

There are limited data on the urinary excretion of different cytokines in hantavirus infection. In a study by Mäkelä and co-workers (2004), plasma IL-6 concentrations and urinary IL-6 excretion were markedly increased in acute NE patients. There was no correlation between plasma and urinary IL-6 levels, probably reflecting the local production of IL-6 in the kidney during acute disease. Furthermore, increased urinary excretion of IL-6 correlated with urinary albumin, IgG and protein excretions, but not with serum creatinine levels (Mäkelä et al. 2004).

Increased production of NO induced by cytokines has been detected in acute PUUV infection (Groeneveld et al. 1995, Linderholm et al. 1996b) as well as in

HCPS (Davis et al. 2002), and may have a role in the pathogenesis of hantavirus infection. However, Borges and co-workers (2008) found no difference in serum levels of NO between HCPS patients and controls, although the levels were higher in fatal HCPS cases compared with survivors. In PUUV infection serum NO levels showed a positive correlation with the severity of the disease and serum creatinine (Groeneveld et al. 1995), and with the degree of hypotension (Linderholm et al. 1996b). Serum NO levels also showed an inverse correlation with blood thrombocyte levels (Groeneveld et al. 1995).

2.3.3.3   Immunohistochemical studies

Immunohistochemical studies of tissue biopsies of patients with hantavirus infection also suggest a role of T lymphocyte activation and cytokines in the pathogenesis of the disease. In kidney biopsies from patients with NE, accumulations of mainly CD8$^+$ T cells have been demonstrated in the peritubular areas (Temonen et al. 1996). Also locally secreted cytokines, including TNF-α, TGF-β and platelet-derived growth factor, have been seen in the peritubular areas of the distal nephron together with enhanced expression of adhesion molecules, including intercellular adhesion molecule-1, vascular cell adhesion molecule, and platelet endothelial cell adhesion molecule (Temonen et al. 1996).

Autopsy studies of HCPS patients have shown that lung tissues contain abundant large immunoblasts consisting of CD4$^+$ and CD8$^+$ T cells (Nolte et al. 1995, Zaki et al. 1995). Mori and co-workers (1999) found high numbers of cells producing monocyte-derived (IL-1α, IL-1β, IL-6 and TNF-α) as well as lymphocyte-derived cytokines (IL-2, IFN-γ, IL-4 and TNF-β) in the spleen and lungs of HCPS patients.

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The number of cytokine-producing cells was only moderately increased in the lungs of patients dying of non-HCPS acute respiratory distress syndrome (ARDS), and very few or no cytokine-producing cells were detected in the lungs of patients who died of causes other than ARDS. Recently, an autopsy study of heart biopsies from HCPS patients showed large macrophages and cardiomyocytes expressing TNF-α protein (Saggioro et al. 2007).

2.3.4   Host genetic factors

There are some data suggesting that the clinical course of HFRS and HCPS is to some extent influenced by host-related immunological factors. In a Finnish study of NE patients, most severe disease was seen in patients who had HLA B8 and DRB1*0301 alleles (Mustonen et al. 1996). All patients suffering from shock had these alleles, and also severe renal failure requiring dialysis treatment was associated with the same alleles. In contrast, only 8 % of 74 NE patients had the HLA B27 allele, and their disease showed a benign clinical course (Mustonen et al. 1998). Kanerva and co-workers (1998b) studied the allelic polymorphism at position −308 of the TNF-α gene promoter region in NE patients and healthy controls, and found that the rarer TNF2 allele, associated with a high TNF-α producer phenotype, was more frequently present in NE patients than in controls. In another Finnish study of NE patients, the clinical course was also more severe in TNF2 allele carriers compared with non-carriers (Mäkelä et al. 2001). However, Mäkelä and co-workers (2002) subsequently showed that the association of the TNF2 allele with severe NE may be due to strong linkage disequilibrium with HLA-B8-DR3. Maes and co-workers (2006) studied the TNF-α gene promoter polymorphism at position

–238 in 36 NE patients, and found patients with the GA-238 genotype (low TNF-α producers) to have a more severe clinical disease course. Further, Paakkala and co-workers (2008) found the presence of HLA alleles B8, DR3 and TNF2 allele to be associated with the severity of abnormal chest radiography findings in NE patients, and especially with the presence of pleural effusion. In a study of NE patients with CNS symptoms, a significant negative correlation between cerebrospinal fluid inflammation and HLA-DR15(2)-DQ6 haplotype was found, indicating a possible role of genetic factors in central nervous system involvement (Hautala et al. 2010).

Data on genetic susceptibility to other hantavirus infections are limited. In a recent study of HCPS patients from Brazil, TNF2 allele was more frequently present in HCPS patients compared to individuals with positive serology for HCPS without a history of clinical disease (Borges et al. 2010). However, there was no difference in the frequency of the TNF2 allele between infected patients and uninfected blood donors, suggesting that this allele does not select individuals who can be infected by hantavirus. Genetic susceptibility to HTNV infection has also been studied, and a significantly higher occurrence of HLA-DRB1*09 and HLA-B*46-DRB1*09 was revealed in Chinese patients compared to controls (Wang ML et al. 2009).

2.4   Long-term prognosis

2.4.1   Renal outcome

The renal outcome of acute hantavirus infection has been considered favorable, although many previous studies have been uncontrolled, with small numbers of patients, or seroepidemiological studies or case reports. The first reports of long-term renal outcome date from the 1960s and 1970s, and thus lack the serological

confirmation of hantavirus infection. Lähdevirta (1971) found no proteinuria in 20 NE patients 1-6 years after acute disease, but renal concentration capacity was decreased in 8 patients and endogenous creatinine clearance in 5. Furthermore, 4 out of the 20 patients had creatinine clearance equally or above 130 ml/min/1.73m^2, indicating glomerular hyperfiltration. Later Lähdevirta and co-workers (1978) reported 5 out of 9 NE patients with slightly depressed tubular function 4-5 years after acute disease, but serum creatinine values and endogenous creatinine clearance were normal in all 9 patients. Earlier Rubini and co-workers (1960) had published a series of 9 HFRS patients of whom 4 developed impaired tubular function over 2 years after the acute phase.

In a prospective study of serologically verified NE cases from Sweden, 3 out of 66 patients had persisting proteinuria (Settergren et al. 1989) and 3 had GFR < 80 ml/min 6 months after acute disease (Settergren et al. 1990). Furthermore, after 8 months of acute disease 3 patients had decreased urine osmolarity (Settergren et al. 1990). However, 2 of the patients with decreased GFR had underlying chronic diseases and one had suffered from severe NE (Settergren et al. 1990). Also 3 patients with tubular dysfunction had chronic diseases which may have contributed to the results (Settergren et al. 1990). A study from Greece, similarly, reported 3 patients with renal tubular acidosis type I (complete in 1 and incomplete in 2 patients) and 2 patients with reduced urine concentrating capacity 1-5 years after acute HFRS (Elisaf et al. 1993). Further, in a Finnish study of 8 NE patients, the urinary excretions and fractional clearances of albumin, IgG and IgG$_4$ remained slightly increased 1 year after acute disease, and ERPF was also significantly lower and FF higher than in healthy controls (Ala-Houhala et al. 2002). Increased urinary

excretion of α1-microglobulin was likewise detected in NE patients 3 months after the acute phase.

Ledina and co-workers (2003) have reported decreased creatinine clearance values in 4 out of 30 (13 %) HFRS patients examined 3-6 years after acute disease. In contrast, increased creatinine clearance was recorded in 10 out of 30 (33 %) patients. Six out of 23 (26 %) patients also had increased urinary excretion of β2-microglobulin, and proteinuria was detected in 11 out of the 23 (48 %) patients. In a register study of patients undergoing quantitative urine protein tests, the prevalence of chronic renal disease was higher in 15 hantavirus seropositive patients compared with 73 seronegative controls (80 % vs. 44 %) (Glass et al. 1990). Glass and co-workers (1993) have also shown that patients with proteinuria or end-stage renal disease are more commonly seropositive for hantavirus infection than the reference group. Furthermore, as a rare phenomenon, a case report from France has described a 15-year old boy with chronic renal failure 30 months after acute NE (Novo et al. 1999), and a report from the United States described two patients with chronic renal failure over 13 months after acute HFRS (Glass et al. 1994).

In a controlled study of the long-term prognosis of NE, Mäkelä and co-workers (2000) retrospectively collected 46 NE patients and compared them with 38 seronegative healthy controls 5 years after the acute phase. Higher GFR and FF were noted in patients than in controls, but there was no difference between ERPF values. Increased glomerular filtration (GFR above 130 ml/min/1.73 m^2) was detected in 13 out of 44 patients (30 %) and in 4 out of 38 controls (11 %). Patients also had higher urinary protein excretion than controls, although the amount of proteinuria could be classified as mild. However, this study added to the evidence of tubular dysfunction several years after acute HFRS, as 9 patients had increased

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overnight urinary excretion of α1-microglobulin compared with only one of the controls showing signs of tubular dysfunction.

Although renal complications during the acute phase of HCPS are not so commonly reported, patients may nonetheless have renal sequelae. In a recent study by Pergam and co-workers (2009), half of their 30 HCPS patients had proteinuria and in 25 % of the patients creatinine clearance was below 90 ml/min/1.73 m^2 from 4 to 96 months after acute disease.

2.4.2 Blood pressure

The first published observations of a possible relationship between hantavirus infection and elevated BP are from HTNV infection in the Korean War. Rubini and co-workers (1960) described 2 out of 13 patients evincing hypertensive vascular disease more than 2 years after acute infection. In a study by Lähdevirta (1971) 7 out of 20 patients had systolic BP over 139 mmHg and 14 had diastolic BP over 89 mmHg 1-6 years after the acute phase. Lähdevirta and co-workers (1978) also reported 1 out of 9 patients as hypertensive 4 years after NE. In a short report by Kleinknecht and Rollin (1994), 2 previously normotensive men needed antihypertensive therapy 2-6 months after acute HFRS. Further, in a retrospective study from Croatia, elevated BP was detected in 9 out of 30 (30 %) patients 3-6 years after HFRS (Ledina et al. 2003).

Seroepidemiological studies from Baltimore have also suggested an association of hantavirus infection with hypertensive renal disease. Hypertensive renal disease was diagnosed in 70 % of 15 seropositive patients compared with 9 % of 73 seronegative patients (Glass et al. 1990). Furthermore, 6.5 % of patients with end-stage renal disease due to hypertension were found to be seropositive for hantavirus

infection (Glass et al. 1993). In a Swedish study comparing 110 PUUV seropositive patients with 682 seronegative patients, hypertension correlated with seropositivity only in 60-year-old individuals, but not in younger patient groups (Niklasson et al. 1994). In contrast with these results, a seroepidemiological study conducted by Ahlm and co-workers (1994a) found no association of hypertension with NE in 83 seropositive patients compared with seronegative controls. In a study by Mäkelä and co-workers (2000), the BP of NE patients were evaluated by ambulatory BP (ABP) measurement. NE patients were found to have higher systolic BP 5 years after acute disease compared with healthy controls. Nine out of 46 (20 %) patients and 2 out of 37 (5 %) were defined as hypertensive after acute NE.

2.5 Hormonal disturbances associated with acute viral infections and acute renal failure

2.5.1 Hormonal abnormalities after acute viral infections

There are a few reports of radiologically confirmed pituitary hemorrhage complicating acute hantavirus infection (Suh et al. 1995, Hautala et al. 2002), and autopsy studies have revealed three other cases (Valtonen et al. 1995). In patients with HTNV infection, repeated brain high-resolution computed tomography scans have shown a progressive decrease in the height of the pituitary gland during a follow-up of 3 months (Lim et al. 1986). Also some reports of an empty sella 1 to 15 years after hantavirus infection have been published (Saltevo and Forslund 1992, Pekic et al. 2005).

There are a number of case reports but no systematic studies concerning pituitary hormonal dysfunction after PUUV infection (Saltevo and Forslund 1992, Settergren

et al. 1992, Hautala et al. 2002, Sane and Färkkilä 2002, Pekic et al. 2005) or other hantavirus infections (Lim et al. 1986, Suh et al. 1995, Park and Pyo 1996, Kim et al. 2001). In the NE case reports, patients suffered from secondary hypothyroidism, hypogonadism and hypocortisolism, i.e. panhypopituitarism. Panhypopituitarism was diagnosed in one patient during the acute phase of NE (Hautala et al. 2002), and in one patient 15 years after the acute disease (Saltevo and Forslund 1992). In the other NE cases, the diagnosis of panhypopituitarism was made 2-9 months after acute NE (Settergren et al. 1992, Hautala et al. 2002, Sane and Färkkilä 2002). Recently, Stojanovic and co-workers (2008) published a retrospective study of 60 patients with previous HFRS from Serbia. A median of two years (mean 3.7) after acute HFRS they found growth hormone deficiency in 13 %, thyroid axis deficiency in 8 %, adrenal axis deficiency in 10 % and gonadal axis deficiency in 12 % of the patients.

There are also some case reports in the literature of other viral infections causing meningitis and encephalitis in adults contributing to hypothalamic-pituitary dysfunction (Hägg et al. 1978, Kupari et al. 1980, Vesely et al. 2000, Schaefer et al. 2008, Tsiakalos et al. 2010). The viruses recognized have been herpes simplex (HSV), Coxsackie B5 (CVB) and other enteroviruses, influenza-A, varicella, and tick-borne encephalitis virus. Evaluation of hypothalamic-pituitary function after viral CNS infections has revealed both panhypopituitarism, including diabetes insipidus and various partial pituitary defects (Schaefer et al. 2008), but the incidence of virus-induced hypopituitarism is unknown.

Several viral infections have also been linked to thyroid diseases (Desailloud and Hober 2009). Retroviruses have been associated with subacute thyroiditis and Graves' disease, the mumps virus with subacute and Hashimoto's thyroiditis, and

the human T lymphotrophic virus-1, enteroviruses, rubella virus, HSV, Epstein-Barr virus (EBV) and parvovirus (PV B19) with Hashimoto's thyroiditis. Furthermore, a recent case report describes a patient with HTNV infection and Graves' disease (Jin et al. 2009). However, it remains obscure whether the viruses are responsible for the thyroid diseases or are merely innocent bystanders (Desailloud and Hober 2009).

2.5.2   Hormonal changes in acute renal failure

Severe acute diseases are known to be associated with a suppression of the hypothalamic-pituitary-gonadal axis (Spratt et al. 1993), or high serum levels of cortisol (Jurney et al. 1987). However, data on possible hormonal dysfunction during acute renal failure are limited.

Kokot and co-workers (1982) found significantly reduced plasma testosterone (Testo) levels and elevated luteinizing hormone (LH) levels, as well as normal follicle-stimulating hormone (FSH) levels in male patients during the oliguric phase of acute renal failure. Furthermore, significantly elevated levels of serum prolactin (PRL) have been reported during acute renal failure. In a report on 20 male patients with acute renal failure caused by hypotension or rhabdomyolysis, the serum levels of FSH and Testo were low during the oliguric and diuretic phase, but normalized after recovery of renal function (Levitan et al. 1984). The serum level of PRL rose during the oliguric phase, but fell to normal during the diuretic phase, and the serum levels of LH did not change during the course of acute renal failure. The responses of FSH and LH to gonadotropin-releasing hormone and of PRL to TSH-releasing hormone were abnormal during acute renal failure, but normalized after recovery of renal function. Uremia per se or its metabolic consequences, for example secondary

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hyperparathyroidism, may have been responsible for these hormonal alterations (Levitan et al. 1984).

In chronic renal failure, the hypothalamic-pituitary-thyroid axis and the peripheral metabolism of thyroid hormones are affected (Iglesias and Diez 2009). Serum levels of thyrotropin (TSH) are usually normal or elevated, and thyroxine (T_4) and T_3 concentrations are normal or low. However, the data on acute kidney injury are limited. In a small study by Kaptein and co-workers (1981), patients suffering from acute renal failure presented with decreased serum levels of T_4 and T_3, and elevated levels of reverse T_3. In a more recent study by Peeters and co-workers (2003), low serum levels of TSH, and T_4 and T_3 were found in critically ill patients. In the subgroup of patients requiring dialysis treatment, the liver enzyme activity contributing to the production of T_3 from T_4 (type I iodothyronine deiodinase) was significantly lower than in patients not requiring dialysis treatment. Tubulointerstitial nephritis with uveitis or drug-induced AIN has also been reported in association with thyroid dysfunction (Iglesias and Diez 2009).

2.6   Glomerulonephritis after viral infections

Viral infections have been recognized to be associated with various glomerular diseases. Diagnosis is usually based on clinical and laboratory data as well as molecular analyses of renal biopsy specimens. Worldwide the most important viral infections relating to glomerulonephritides are hepatitis B (HBV) and C (HCV) infections. HBV infection has been mainly associated with membranous GN (MGN), membranoproliferative GN (MPGN) and IgAN (di Belgiojoso et al. 2002). As regards HCV infection, the cryoglobulinemia-mediated MPGN is the most common, but also MPGN without cryoglobulinemia, as well as MGN and IgAN

have been diagnosed (di Belgiojoso et al. 2002). Furthermore, human immunodeficiency virus (HIV) infection is especially closely related to the collapsing form of focal segmental glomerulosclerosis known as HIV-associated nephropathy (di Belgiojoso et al. 2002).

In 1990, Grcevska and co-workers published a report on a patient with HTNV infection and diffuse proliferative glomerulonephritis with complete recovery of renal function. In a Finnish renal biopsy material of 86 acute NE patients, there was one case with IgAN (Mustonen et al. 1994b). In both of these cases the association of hantavirus infection with glomerulonephritis may be incidental. Recently, a case report of crescentic glomerulonephritis in a patient with HTNV infection and a 3 months' period of dialysis treatment was published (Kim et al. 2010). However, the association of glomerulonephritis with hantavirus infection in this case has been questioned by the reason of the atypical clinical course and lack of serological confirmation of recent HFRS (Clement et al. 2011).

Previously, Mustonen and co-workers (2001) reported 5 patients with MPGN emerging during the convalescent phase of acute NE. All 5 patients suffered from peripheral swellings shortly after NE. At the time of renal biopsy, the amount of daily urinary protein excretion varied from 8 to 16 grams, and urinary sediment also revealed hematuria. Renal insufficiency was noted in 4 out of 5 patients with serum creatinine varying from 122 µmol/l to 524 µmol/l, and 4 patients were hypertensive. Three patients were treated with oral methylprednisolone. Four patients went into remission, but one started chronic dialysis treatment 3 months after the MPGN diagnosis. Renal biopsies were performed on 5 patients from 3 to 8 weeks after serologically verified acute NE. The light-microscopy finding was typical MPGN type 1 in all 5 cases, and in the specimens from 4 patients, there were infiltrating

polymorphonuclear leukocytes giving the exudative feature. In 4 cases IF specimens were available and showed granular and diffuse deposits of complement C1q or C3 mainly in the capillary walls, and IgA, IgG and IgM were also found in the glomeruli.

Other viruses are also possibly associated with glomerular diseases (di Belgiojoso et al. 2002). Hepatitis A virus has been reported with diffuse proliferative GN, PV B19 with collapsing glomerulopathy, endocapillary and mesangioproliferative GN, and cytomegalovirus (CMV), CVB as well as EBV have been suspected to be involved in the pathogenesis of IgAN. Recent case reports also suggest an association of CMV with MGN (Georgaki-Angelaki et al. 2009) and of EBV with MPGN (Karamadoukis et al. 2008).

3. AIMS OF THE STUDY

The aims of this study were:

1. To establish the long-term outcome of nephropathia epidemica (I and III)

2. To study whether the clinical severity of nephropathia epidemica, as well as certain host genetic factors, predict the long-term outcome (II)

3. To determine the prevalence of hormonal defects in patients with acute nephropathia epidemica and during long-term follow-up (IV)

4. To describe seven patients presenting with nephrotic syndrome shortly after acute nephropathia epidemica (V)

4. SUBJECTS AND METHODS

4.1 Subjects

4.1.1 Patients

Studies I, II and IV originally involved 70 prospectively recruited consecutive patients with acute hospital-treated NE during the years 1997-1999 (Figure 2). They were examined during the acute phase of the condition, and invited to attend a follow-up visit 3 and 12 months after acute disease. Fifty patients attended the 3-month and 56 the 12-month visit. A third follow-up visit was scheduled 4-7 years after acute disease in 2004, and 43 patients participated.

Thirteen out of these 43 patients had had chronic diseases prior to acute NE: hypertension in four, hypothyroidism in three, and diabetic nephropathy, glomerulonephritis, celiac disease, coronary heart disease, bronchial asthma and ankylosing spondylarthritis in one each. Henoch-Schöenlein purpura had been diagnosed in one patient over 30 years previously and renal tuberculosis in one patient likewise 30 years previously, but neither one showed any signs of chronic renal disease during the examinations.

Since the aim of study I was to establish whether NE induces alterations in renal function and blood pressure, patients with a history of hypertension, diabetic nephropathy, and glomerulonephritis prior to NE were excluded from that study. Study I thus comprised altogether 37 patients (29 males and 8 females) aged 29-70 (mean 49). The same cohort was included in study II. Neither the clinical severity of

acute NE nor the age of 37 patients participating in studies I and II differed from the 33 dropouts of the original cohort (data not shown).

Study IV involved 54 out of those 56 patients who participated in the 12-month visit, and whose serum samples were available for retrospective hormonal analyses. The cohort comprised 37 males and 17 females, aged from 15 to 70 (median 42) years (Figure 2). The severity of clinical picture of acute NE of 54 patients did not differ from the 16 dropouts of the original cohort (data not shown). However, these 54 patients were slightly older than the dropouts during the acute NE (43±13 vs. 35±11 years, P=0.038).

Forty-six patients attended the 3-month visit. When the third prearranged follow-up visit was scheduled in 2004, 38 out of these 54 patients attended. All serum hormonal levels were analysed retrospectively in 2007. On the basis of the results, six patients were invited to make an additional visit in 2008 (9 to 10 years after NE). The following chronic diseases were recorded in 14 patients: hypertension in four and hypothyroidism in two, as well as ankylosing spondylarthrosis, bronchial asthma, aortic valve disease, celiac disease, sequelae of renal tuberculosis, operated cancer of the vocal cords, operated meningeoma and osteoporosis in one each.

The cohort for study III consisted originally of 46 patients hospitalized because of acute NE during the years 1990–1995 in Tampere University Hospital (Figure 2). Participants were recruited retrospectively. Thirty-six patients, 20 males and 16 females, were examined 3 to 7 (mean 5) years after acute NE during the period 1996 to 1997, and for a second time in 2003, 8 to 13 (mean 10) years after acute disease. In 2003, they were aged from 32 to 68 (mean 50) years. Five out of the 36 patients had suffered from some chronic disease before NE: ankylosing spondylarthritis (two patients), as well as coronary heart disease, bronchial asthma and chronic

schizophrenia in one each. The patient with asthma also had hypothyroidism. Data on the 5-year prognosis of this cohort of 46 patients have been published earlier (Mäkelä et al. 2000).

Study V involved 7 patients (4 females and 3 males, aged from 27 to 59 years) with biopsy-proven glomerulonephritis shortly after acute NE (Figure 2). They suffered from acute NE during the years 2003-2008, except for one patient in 1992. One female patient had type I diabetes without complications, and two female patients had used L-thyroxine substitution for hypothyroidism before acute NE. None had used antihypertensive therapy before NE. The patients were from four different health care districts in Finland: four from Mikkeli Central Hospital and one each from Päijät-Häme Central Hospital, Länsi-Uusimaa Hospital and Tampere University Hospital. The physicians had diagnosed, treated and followed the patients in their own hospitals.

In studies I-IV all patients continued their regular medication during the studies. Three patients in studies I and II, and two in study IV received L-thyroxine substitution therapy, but none received corticosteroid therapy.

4.1.2 Controls

Thirty-eight healthy controls with negative PUUV-serology had been recruited in a previous study of the 5-year prognosis of NE during the years 1996 to 1998 (Mäkelä et al. 2000). There were 22 males and 16 females aged from 27 to 64 (mean 44) years. The results of these examinations were used in study I. Controls were volunteers collected by open advertisement in the notice board of Tampere University Hospital. They were matched according to age and body mass index

(BMI) with the patients of the previous study of the 5-year prognosis of NE (Mäkelä et al. 2000).

In 2003, a follow-up visit was scheduled. Twenty-nine controls, 14 males and 15 females aged from 30 to 69 (mean 51) years, took part. They comprised the control group for study III. One of the controls in studies I and III suffered from hypothyroidism and continued her L-thyroxine substitution therapy during the examinations.

Figure 2. Description of the patient groups of the studies I-V.
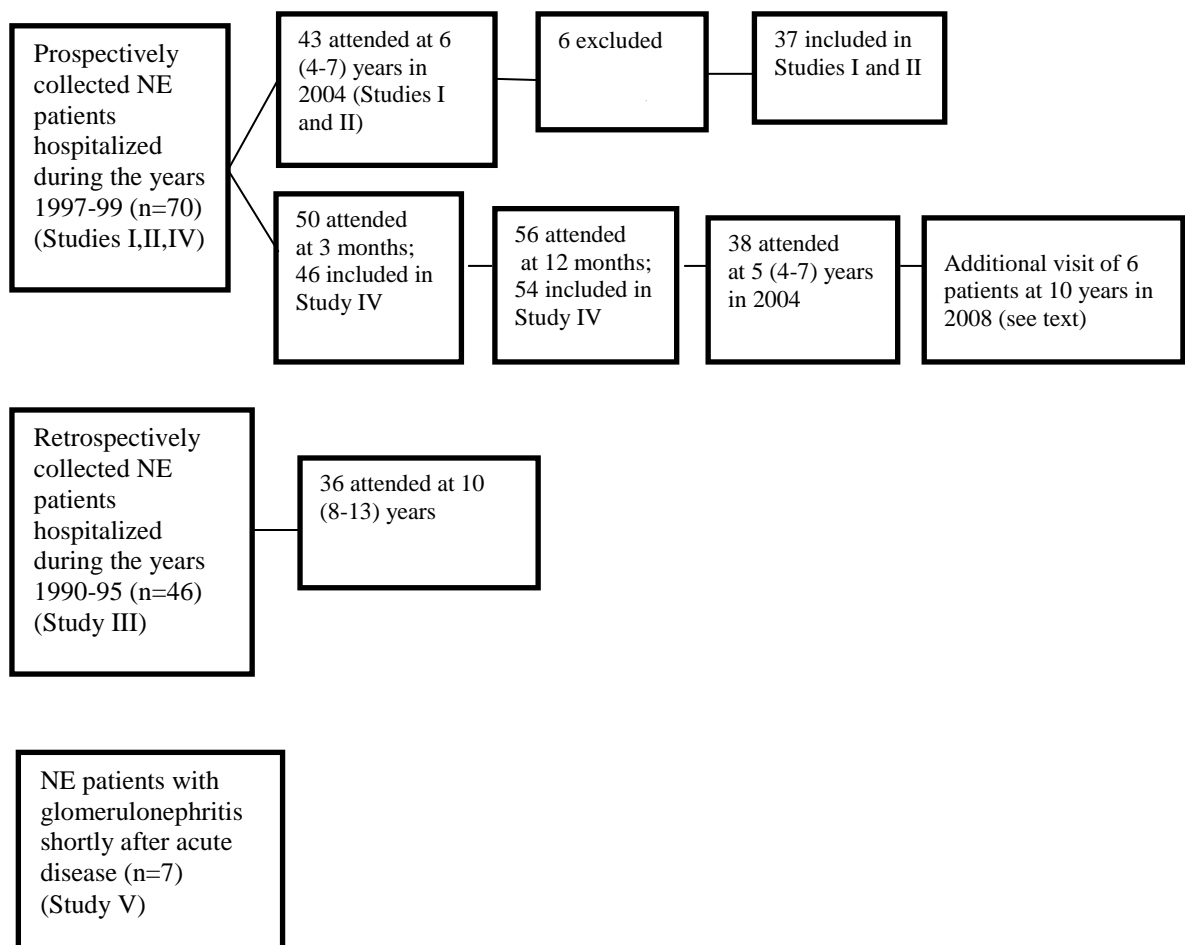
4.2   Methods

4.2.1   Study protocols

During the acute phase of NE (studies I, II and IV), detailed medical histories of the patients were obtained and physical examination performed. All blood specimens were drawn between 7.30 and 9.30 in the morning. The blood samples for analysis of plasma CRP, IL-6 and TNF-α, serum creatinine and blood cell count, as well as 24-hour urinary protein and overnight α1-microglobulin, were collected on three consecutive mornings after hospital admission. The first blood sample for hormonal analyses in study IV was obtained on the first morning of hospital care, the second was taken on the third morning. The highest and the lowest value for the various variables measured during hospitalization were designated as maximum and minimum values, respectively. All serum samples for hormone analyses in study IV were stored at -70 ºC until analyzed.

   All the follow-up visits in studies I-IV were made on an outpatient basis in Tampere University Hospital. All blood samples for these visits were obtained in the morning after a minimum 12-hour fast, as were the spot samples of the morning urine.

4.2.2   Analytical methods

Plasma CRP levels were analyzed by Hitachi 705E analyzer from 1997 to 1998 and thereafter by a Roche Diagnostics CRP method using a Cobas Integra analyzer (F Hoffmann-LaRoche Ltd, Basel, Switzerland). The blood cell count was carried out by H2 or H3 hematological analyzer (Bayer Corporation, Tarrytown, NY, USA). From 1990 to 1999 serum creatinine, cholesterol and blood glucose were

determined by Vitros (Johnson&Johnson, Rochester, NY, USA), and from 2003 by Cobas Integra analyzer. Serum creatinine concentrations showed 10 % lower values during the years 2003 and 2004 than from 1990 to 1999 due to this change in determination method. The results on serum creatinine concentrations during the years 1990 to 1999 were multiplied by the coefficient 0.9 (studies I-III). Fasting blood glucose was determined from whole blood until the year 2003, whereafter the analysis was performed in plasma, thus producing 12 % higher values. The results for fasting plasma glucose before the year 2003 were multiplied by the coefficient 1.12 (study I). In study I, the limits for impaired fasting glucose (IFG) were 6.1-6.9 mmol/l, according to the guideline of the European Diabetes Epidemiology Group (Forouhi et al. 2006).

In study IV all serum hormonal levels were analyzed retrospectively in 2007. Serum free thyroxine (fT4) and TSH were measured by a chemiluminescent microparticle immunoassay (Abbott Architect i2000 system, Abbott laboratories, IL, USA), cortisol, PRL, LH, and FSH by fluoroimmunoassay (1235 AutoDELFIA, Wallac Ltd., Turku, Finland), and estradiol (E2) as well as Testo by radioimmunoassay (1277 Gammamaster, Wallac Ltd). Serum levels of growth hormone (GH) were determined by time-resolved fluoroimmunoassay (AutoDELFIA hGH, Wallac Ltd.) and antibodies against thyroid peroxidase (TPOAb) by immunoluminometric assay (Abbott Architect Anti-TPO, Wiesbaden, Germany).

The reference ranges in study IV were 9.0-19.0 pmol/l for fT4, 0.4-4.0 mU/l for TSH, 180-680 nmol/l for cortisol, < 450 mU/l for PRL in male patients, < 600 mU/l in premenopausal women, and < 280 mU/l in postmenopausal women. The male reference ranges were 10.4-34.6 nmol/l for Testo, and 0.7-6.7 U/l for LH. As the

phase of the menstrual cycle was not known, E2 below 0.1 nmol/l was regarded as a hypogonadal value for a premenopausal woman. The GH reference range was 0-11.5 mU/l and for TPOAb <6 U/ml.

Peri- and postmenopausal women in study IV were not analyzed for defects of the gonadal axis, as they were hypogonadal by definition. Their FSH and E2 levels were, however, included in the analysis of hormonal alterations between the acute phase of NE and 3 months later. Chronic, overt hypogonadism was defined as Testo (in men) and E2 (in premenopausal women) repeatedly below the reference range one year or more after acute NE. Chronic, subclinical/compensated testicular failure was diagnosed in male patients with chronically elevated LH but Testo within the reference range. Chronic, overt hypothyroidism was defined as fT4 repeatedly below 9.0 pmol/l and/or TSH repeatedly above 4.0 mU/l in patients with chronic thyroiditis, goiter, and/or symptoms suggestive of hypothyroidism (Baskin et al. 2002).

The 24-hour urinary protein excretion were measured by the pyrogallolal red molybdate method (Olli C, Kone Instruments, Helsinki, Finland) in the 1990s and by Cobas Integra 700/800 analyzer in 2003-2004 (studies I-III). The limit for increased 24-hour protein excretion was ≥ 0.22 g (study II). The overnight urinary excretion of α1-microglobulin was measured by nephelometry (Behring Nephelometer II analyzer, Behringwerke AG, Marburg, Germany) (studies I-III). A value for overnight urinary excretion of α1-microglobulin ≥ 7 μg/min was considered abnormal, based on the reference values for healthy subjects from the laboratory of Tampere University Hospital (studies I-III).

4.2.3 Puumala virus serology

The serology of PUUV infection was detected by analyzing IgM- and IgG-class antibodies by enzyme immunoassays (Vapalahti et al. 1996b) (studies I-V). This analysis is based on the specific reaction of sera with PUUV NP. In study V, the PUUV infection in 3 out of 7 patients was diagnosed by rapid immunochromatographic IgM-antibody test (Hujakka et al. 2001).

4.2.4 Cytokines

Plasma IL-6 and TNF-α concentrations in studies II and IV were determined using commercially available enzyme-linked immunosorbent assays (ELISA; PeliKine Compact^{TM} human IL-6 and TNF-α kits; Red Cross Blood Transfusion Service, Amsterdam, the Netherlands). The detection limits for the assays were 0.4 pg/mL for IL-6 and 1.4 pg/mL for TNF-α. In the calculations, a concentration less than the detection limit was regarded as the detection limit of the assay in question. The highest plasma cytokine concentration determined during the first 3 days of hospitalization was designated the maximum cytokine concentration and used in calculations.

4.2.5 Cytokine gene polymorphism and human leukocyte antigens

The analyses of TNF-α and IL-6 gene polymorphism in study II were performed as described elsewhere (Wilson et al. 1992, Hulkkonen et al. 2001); the analyses of HLA-B8, -DR3 and -B27 in studies II and IV were determined as described by Westman and co-workers (1993).

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4.2.6 Blood pressure

Twenty-four-hour ABP was measured in studies I-III during one 24-hour period by oscillometric and/or auscultatory method using a non-invasive, fully automatic recorder (Novacor Diasys Integra; Novacor SA, Rueil-Malmaison France). Measurements were recorded at 15-minute intervals during the daytime, and at 30-minute intervals during the night. The cut-off point for ABP used to define hypertension was a 24-hour mean ABP of more than 133/82 mmHg with reference to a large population study (Staessen et al. 1994).

In studies II and III, the patients were defined as hypertensive if they were on hypertensive therapy, had a mean ABP ≥133/82 mm/Hg, or office BP ≥140/90 mm/Hg (Guidelines Sub-Committee, 1999). Office BP was used to define hypertension only if ABP monitoring was not determined.

4.2.7 Glomerular filtration rate and effective renal plasma flow

In studies I-III, GFR was determined by a single-injection method as the plasma clearance of ^{51}Cr-EDTA after a light meal, and expressed in values normalized for body surface area. Based on studies in healthy subjects, increased GFR was defined as a GFR $\geq$ 130 ml/min/1.73 m^2 (studies I and II) (Vervoort et al. 2005). ERPF was estimated by the clearance of ^{131}I-hippurate (studies I-III).

4.2.8 Renal biopsy

In study V, the renal biopsy specimens were processed for light-microscopic and IF studies. Paraffin sections were stained by hematoxylin-eosin, periodic acid/Schiff, Masson's trichrome and Jones' periodic Schiff-methenamine methods. IF studies

were made on cryostat sections using FITC-conjugated antisera against human IgG, IgA, IgM, C1q, C3, fibrinogen as well as kappa and lambda.

4.2.9 Radiological examinations

In study IV, thyroid ultrasound imaging (Vivid I, GEMS Ultrasound, Tirat Carmel, Israel) was performed by the same investigator (P.J.) on the 6 patients attending the additional visit in 2008. The hypothalamic-pituitary region was studied by MRI (MAGNETOM Trio 3-Tesla scanner, Siemens, Erlangen, Germany) in patients showing distinct hormonal deficits of central origin.

4.2.10 Mathematical formulas

Body mass index was calculated by dividing weight (in kilograms) by the square of height (in square meters) (studies I and III). FF in studies I and III was calculated as the quotient of GFR and ERPF.

4.2.11 Statistical methods

To describe data from studies I-IV, means and standard deviations (SDs) are given for normally distributed variables, and medians and ranges for skew-distributed continuous variables. In studies I-III, groups were compared using independent sample t-tests for normally distributed continuous variables, and Mann-Whitney U tests for skewed variables (studies I-IV). Categorical data were analyzed by χ^2 test or Fisher's exact test (studies I, III-IV). In study II, patients were divided into 2 groups as having or not having elevated excretion of α1-microglobulin, increased GFR or hypertension, respectively. The Wilcoxon signed-rank test was used in

study IV to evaluate changes in hormone levels between the acute phase and 3 months afterwards, and between 3 months and 1 year or 5 years, respectively. Correlations were analyzed using Pearson's correlation coefficient (study I) and by Spearman's rank correlation coefficient (study IV). Skew-distributed urinary excretion of protein was log-transformed before analysis in study I. Binary logistic regression analysis was used in study I to describe the possible influence on results of differences in age and gender distribution between the patient and control groups. Odds ratios and their 95 % confidence intervals were given (study I). All tests were two-sided, and a p-value less than 0.05 was considered statistically significant. All analyses were performed with the SPSS for Windows (SPSS Inc., Chicago, IL, USA).

4.3 Ethical considerations

Written informed consent was obtained from all patients and controls participating to studies I-IV. For study V, the physician taking care of the patient asked patients' permission to evaluate their hospital records. Studies I-IV were approved by the Ethics Committee of Tampere University Hospital.

5. RESULTS

5.1 Outcome of patients six years after acute nephropathia epidemica and the influence of the clinical severity of acute disease on the long-term prognosis (Studies I and II)

Patients had higher mean systolic BP, GFR and daily urinary excretion of protein than controls six years after acute NE (Table 4). Further, mean night-time systolic and diastolic BP values were likewise higher in patients than in controls [109±11 (mean±SD) vs. 100±9 mmHg, P=0.001 and 70±7 vs. 66±7 mmHg, P=0.035, respectively]. Five patients had received antihypertensive medication after acute NE, and they continued their medication during the study, while none of the controls used antihypertensive drugs. Altogether 10 out of 37 (27 %) patients were found to be hypertensive after acute NE. GFR ≥130 ml/min/1.73m^2 was detected in 10 out of 37 (27 %) patients compared with 4 out of 38 (11 %) controls (P=0.067). ERPF and FF did not differ between the patient and control groups (646±116 vs. 627±105 ml/min/1.73m^2, P=0.470 and 19±3 vs. 18±3 %, P=0.166, respectively). Increased urinary excretion of α1-microglobulin was detected in 9 out of 35 (26 %) patients compared with 1 out of 38 (3 %) controls (P=0.005).

Patients were approximately five years older than controls, and their serum low-density lipoprotein cholesterol and fasting blood glucose levels were higher than in controls (Table 4). BMI or serum creatinine values did not differ between the groups. Type 2 diabetes had been diagnosed in one male patient after acute NE, and

one female patient was found to have high fasting blood glucose during the study. IFG was found in 7 out of 37 (19 %) patients, but in none of the controls (P=0.005).

After adjusting for age and sex, the differences between the groups in BP, GFR and urinary protein excretion remained ($P < 0.05$ in each). GFR correlated inversely with age in the patient group, but not in the control group (r=─0.512 (correlation coefficient), P=0.001 and r=0.008, P=0.963, respectively). GFR was essentially the same in patients with diabetes or IFG compared with those with normal blood glucose values (118±15 vs. 119±20 ml/min/1.73 m^2, P=0.194). Proteinuria and GFR were also positively correlated in the patient group (r=0.495, P=0.003), but proteinuria did not correlate with age or BP in either group (data not shown).

Table 4. Clinical and laboratory findings in patients six years after acute NE compared with healthy controls.

Finding	Patients (n=37)	Controls (n=38)	P value
Males/females (percentages of males)	29/8 (78 %)	22/16 (58 %)	NS
Age (years)	49 ± 11	44 ± 10	0.042
Body mass index (kg/m^2)	26 (20-47)	25 (21-34)	NS
24-hour mean systolic BP (mmHg)[a]	123 ± 11	117 ± 9	0.012
Serum creatinine (µmol/l)	70 ± 12	73 ± 11	NS
GFR (ml/min/1.73m^2)	119 ± 19	109 ± 14	0.016
Proteinuria (g/day)[b]	0.17 ± 0.05	0.14 ± 0.04	0.006
Fasting blood glucose (mmol/l)	5.7 ± 0.6	5.1 ± 0.5	<0.001
Serum LDL cholesterol (mmol/l)	3.5 (2.4-5.3)	3.2 (2.2-4.7)	0.039

Means±SD are given for normally distributed variables and medians (range) for skew-distributed variables.

Abbreviations: BP=blood pressure, GFR=glomerular filtration rate.

[a]ABP monitoring was completed in 27 patients and 37 controls.

[b]Urine for measurement of proteinuria was collected from 34 patients.

Tables 5 and 6 show the relation of variables reflecting the clinical severity of acute NE to the presence of urinary excretion of α1-microglobulin (Table 5), as well as to the presence of increased GFR and hypertension (Table 6) six years after acute disease. The results imply that abnormal findings found at six-year examinations cannot be predicted by the clinical course of acute NE. However, the maximum plasma IL-6 level during the acute phase of NE was higher in patients with increased urinary excretion of α1-microglobulin at six years than in patients with no excretion of α1-microglobulin (Table 5). Neither the plasma levels of IL-6 nor TNF-α during the acute phase had any relation to the daily urinary excretion of protein, GFR or hypertension six years later (data not shown). Furthermore, the HLA-B8, -DR3, -B27 alleles, or TNF-α(-308) allele A or IL-6(-174) allele G were not related to renal function, urinary excretion of protein or hypertension six years after acute NE (data not shown).

Table 5. Acute-phase laboratory findings in patients with increased or normal urinary excretion of α1-microglobulin six years after NE.

Finding during the acute phase of NE	Urinary excretion of α-microglobulin 6 years after acute NE		
	Increased (n=9)*	Normal (n=26)	*P* value
B-Throm$_{min}$ (10^9/l)	52 ± 26	68 ± 32	0.190
S-CRP$_{max}$ (mg/l)	84 (50-213)	64 (20-175)	0.128
S-Crea$_{max}$ (μmol/l)	242 (81-653)	241 (70-878)	0.725
dU-Protein$_{max}$ (g/day)	1.78 (0.71-17.78)	1.68 (0.30-10.00)	0.697
cU-α1-miglo$_{max}$ (μg/min)	37 (11-46)	27 (7-209)	0.725
P-IL-6$_{max}$ (pg/ml)	25.0 (14.8-59.1)	14.0 (3.3-43.7)	0.038
P-TNF-α$_{max}$ (pg/ml)	7.0 (1.8-16.3) (n=6)	5.7 (1.6-37.4) (n=19)	0.975

Means±SD are given for normally distributed variables and medians (range) for skew-distributed variables. Abbreviations: B-Throm$_{min}$=lowest blood platelet count, S-CRP$_{max}$=highest serum C-reactive protein concentration, S-Crea$_{max}$=highest serum creatinine, dU-Protein$_{max}$=highest daily urinary protein excretion, cU-α1-miglo$_{max}$=highest overnight urinary α1-microglobulin, P-IL-6$_{max}$=highest plasma interleukin-6, P-TNF-α$_{max}$=highest plasma tumor necrosis factor. *≥ 7 μg/min

Table 6. Acute-phase laboratory findings in patients with increased or normal GFR, and in those with hypertension or normotension six years after acute NE.

Finding during the acute phase of NE	GFR 6 years after acute NE			Hypertension 6 years after acute NE		
	Increased (n=10)	Normal (n=27)	P value	Present (n=10)	Absent (n=27)	P value
B-Throm$_{min}$ (10^9/l)	60 ± 32	64 ± 32	0.770	75 ± 28	59 ± 33	0.192
S-CRP$_{max}$ (mg/l)	80 (20-175)	72 (22-213)	0.656	100 (22-213)	69 (20-157)	0.098
S-Crea$_{max}$ (µmol/l)	285 (78-626)	192 (70-878)	0.906	321 (70-878)	187 (77-749)	0.335
dU-Protein$_{max}$ (g/day)	1.74 (0.14-10.00)	1.66 (0.30-17.78)	0.801	1.75 (0.30-4.85)	1.66 (0.14-17.78)	0.555
cU-α1-miglo$_{max}$ (µg/min)	24 (7-209)	29 (11-137)	0.511	29 (11-209)	26 (7-137)	0.371

Means±SD are given for normally distributed variables and medians (range) for skew-distributed variables. Abbreviations: B-Throm$_{min}$=lowest blood platelet count, S-CRP$_{max}$=highest serum C-reactive protein concentration, C-Crea$_{max}$=highest serum creatinine, dU-Protein$_{max}$=highest daily urinary protein excretion, cU-α1-miglo$_{max}$=highest overnight urinary α1-microglobulin.

5.2 Ten-year prognosis of acute NE (Study III)

Compared to controls patients had higher mean ambulatory systolic and diastolic BP (Figure 3) as well as daytime and night-time systolic BP (data not shown) five years after acute NE. At ten years there was no difference between the groups either in mean ambulatory systolic and diastolic BP (Figure 4) or in daytime and night-time BP (data not shown). However, according to the definition applied for the prevalence of hypertension there was a trend toward more hypertensive subjects in the patient group than in the control group at 10 years (39 % vs. 17 %, *P*=0.098).

Table 7 shows the results on daily urinary protein excretion and nightly excretion of α1-microglobulin 5 and 10 years after acute NE in patients compared with healthy controls. There was more proteinuria in patients than controls at 5 years, but

not at 10 years. There were also higher percentages of patients than controls at 5 years evincing elevated urinary excretion of α1-microglobulin, but the difference did not reach statistical significance. GFR was higher in patients than in controls at 5 years (121±19 vs. 109±16 ml/min/1.73 m^2, P=0.012), while at 10 years patients' GFR had decreased to the same level as in controls (113±20 vs. 108±17 ml/min/1.73 m^2, P=0.370). There were no statistically significant differences between the groups in ERPF or FF 5 and 10 years after NE (ERPF at 5 years: 627±105 vs. 615±112 ml/min/1.73 m^2, P=0.652, FF at 5 years: 20±3 vs. 18±3 %, P=0.071 and ERPF at 10 years: 606±115 vs. 592±109 ml/min/1.73 m^2, P=0.623, FF at 10 years: 19±4 vs. 19±3 %, P=0.670).

Patients had 1.8 units higher BMI at 10 years than at 5 years, whereas BMI among controls had not changed [27 (19-48) (median (range)) vs. 29 (22-44) kg/m^2 and 26 (21-34) vs. 26 (21-33) kg/m^2, respectively], and the differences in BMI between patients and controls at 10 years were significant (P=0.003). Fasting blood glucose did not differ between the groups at 5 years (4.5±0.5 vs. 4.5±0.4 mmol/l, P=0.838), but was higher in patients at 10 years (5.1±0.6 vs. 4.6±0.4 mmol/l, P=0.002).
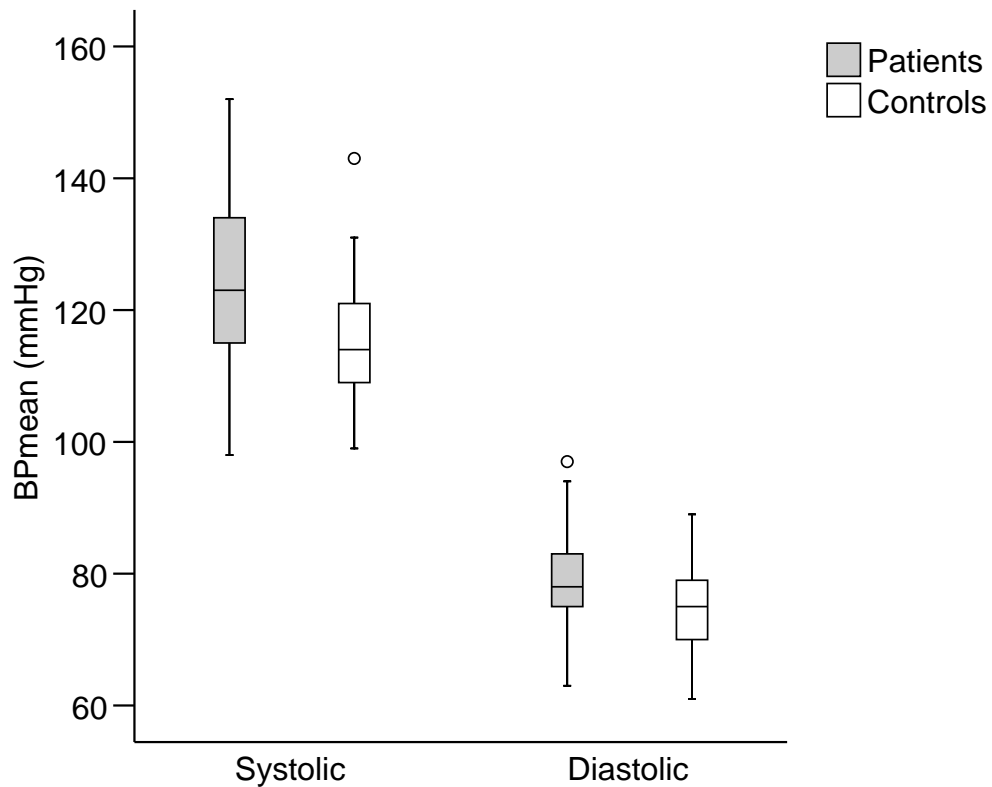
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Figure 3. The mean ambulatory systolic and diastolic BP in patients 5 years after acute NE compared with healthy controls. Box plots of mean ambulatory systolic and diastolic BP show the median (─), the 25% and 75% percentiles (boxes), and the lowest and highest values (whiskers) which are not outliers. The outliers in mean systolic BP in controls and mean diastolic BP in patients are indicated by open circles. Outliers are the values which are between one and a half and three box lengths from either end of the box. The P values for both systolic and diastolic BP are < 0.05. Abbreviations: BP=blood pressure.

Figure 4. The mean ambulatory systolic and diastolic BP in patients 10 years after acute NE compared with healthy controls. Box plots of mean ambulatory systolic and diastolic BP show the median (─), the 25% and 75% percentiles (boxes), and the lowest and highest values (whiskers) which are not outliers. The outliers in mean systolic BP in controls and patients are indicated by open circles. Outliers are the values which are between one and a half and three box lengths from either end of the box. The P values for both systolic and diastolic BP are non-significant. Abbreviations: BP=blood pressure.
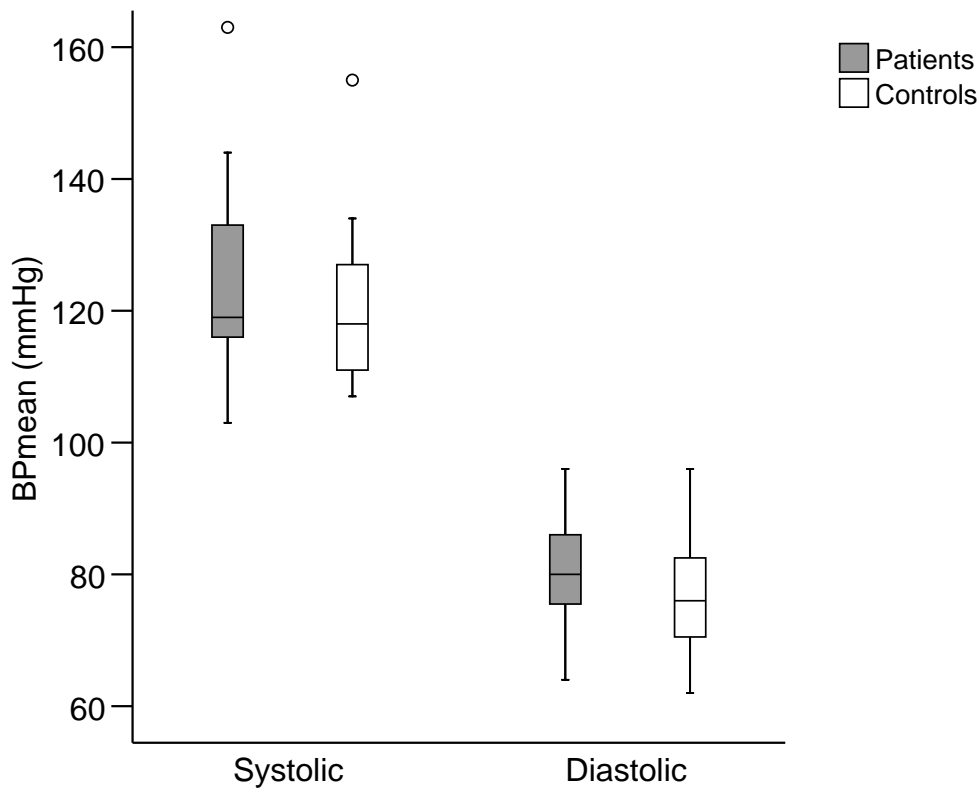
Table 7. Daily urinary protein excretion and the prevalence of nightly urinary α1-microglobulin excretion in patients five and ten years after acute NE compared with healthy controls.

	Patients (n=34-36)	Controls (n=29)		Patients (n=36)	Controls (n=29)	
Study year	1998 (5 years)	1998	*P* value	2003 (10 years)	2003	*P* value
UPE (g/day)	0.19 (0.12-0.38)	0.14 (0.09-0.24)	<0.001	0.14 (0.07-0.24)	0.13 (0.06-0.31)	0.610
α1-miglo (yes/n)[a]	7/34 (21 %)	1/29 (3 %)	0.060	4/36 (11 %)	2/29 (7 %)	0.684

Medians (range) are given for skew-distributed variables.

Abbreviations: UPE=urinary protein excretion, α1-miglo=α1-microglobulin.

[a]Yes=urinary excretion of α1-micro ≥7 µg/min.

5.3   Hormonal changes associated with NE (Study IV)

5.3.1   Hormonal changes during acute NE and 3 months later

Altogether 30 out of 54 (56 %) patients evinced abnormalities of the gonadal and/or thyroid axis during acute NE. The itemized prevalences of these abnormalities are shown in Table 8. Altogether 22 out of 54 (41 %) had multiple hormonal abnormalities. One of the five patients with elevated TSH and low-normal fT4 levels, had been diagnosed with primary hypothyroidism previously and was using L-thyroxine substitution during the study.

Central hormonal defects were detected in 19 and primary defects in 13 patients. Those with central hormonal defects had higher plasma creatinine concentrations and blood leukocyte counts compared with those with normal hormonal levels

(creatinine: 368 (79-749) vs. 208 (76-878) µmol/l, *P*=0.029 and blood leukocyte count: 13.4 (4.4-22.8) vs. 9.2 (6.7-23.4) x10^9/l, *P*=0.033). The clinical picture of acute NE in patients with primary hormonal deficiencies did not differ from those with normal hormonal levels (data not shown). Neither were the cytokine levels nor HLA-B8, -DR3 and -B27 alleles related to the primary hormonal defects (data not shown).

Table 8. Prevalence of gonadal and thyroid hormone deficiencies during acute NE.

Group of patients	Males (n=37)		Premenopausal women (n=6)	All patients (n=54)	
Serum hormone level abnormalities	Low Testo	Elevated LH with normal Testo	Low E2 and FSH	Low fT4 and low-normal TSH	Elevated TSH and low-normal fT4
Number of patients (%)	18/37 (49 %)	5/37 (14 %)	3/6 (50 %)	2/54 (4 %)	5/54 (9 %)

Abbreviations: Testo=testosterone, LH=luteinizing hormone, E2=estradiol, fT4=free thyroxine, TSH=thyrotropin.

In males, serum Testo levels were lower during acute NE compared with the levels 3 months later (Figure 5A) (9.9 (1.1-27.0) vs. 17.3 (7.8-25.4) nmol/l), whereas there was no difference in serum LH levels (Figure 5B) (4.0 (0.9-18.0) vs. 3.6 (2.0-7.9) U/l). In females, serum FSH levels were lower during acute NE compared with those measured 3 months later (4.7 (0.6-40.4) vs. 23.7 (3.0-73.2) U/l, *P*=0.001), but no differences were noted in E2 levels (0.07 (0.02-0.42) vs. 0.11 (0.04-0.49) nmol/l, *P*=0.263). Serum levels of cortisol (Figure 6) and PRL (Figure

7) were higher in both females and males during the acute phase than 3 months later (cortisol: 506 (358-928) vs. 305 (163-606) nmol/l in females and 551 (267-1157) vs. 376 (182-553) nmol/l in males, prolactin: 269 (102-1364) vs. 212 (84-550) mU/l in females and 247 (32-829) vs. 124 (46-270) mU/l in males). Likewise, TSH was higher during acute NE compared with the serum levels 3 months later in the whole study group (1.90 (0.10-9.00) vs. 1.70 (0.02-7.60) mU/l, $P=0.011$), whereas the serum fT4 levels did not differ (data not shown).

In males, the minimum serum Testo levels correlated inversely with age and blood leukocyte count ($r=-0.341$, $P=0.039$, and $r=-0493$, $P=0.002$, respectively), and with maximum levels of serum creatinine, IL-6, CRP and PRL ($r=-0.437$, $P=0.007$; $r=-0.411$, $P=0.011$; $r=-0.352$, $P=0.032$; $r=-0.363$, $P=0.027$, respectively), but not with BMI ($r=-0.009$, $P=0.957$). In the whole study group, the serum cortisol and PRL levels during the acute NE correlated positively with maximum plasma creatinine ($r=0.352$, $P=0.009$ and $r=0.511$, $P<0.001$, respectively) as well as IL-6 levels ($r=0.319$, $P=0.019$ and $r=0.338$, $P=0.012$), and with blood leukocyte count ($r=0.370$, $P=0.006$ and $r=0.421$, $P=0.002$). Serum cortisol levels also correlated positively with serum PRL levels during the acute phase of NE ($r=0.402$, $P=0.003$). Serum fT4 and TSH levels measured during acute NE did not correlate with age, BMI, plasma creatinine, CRP or cytokine levels (data not shown).

Figure 5. Serum Testo levels (A) and serum LH levels (B) in males during acute NE and after three months. The serum Testo levels were lower during acute NE than 3 months after, whereas serum LH levels did not differ between the acute phase and 3 months after. (*P*<0.001 for Testo and *P*=0.399 for LH; reference range for Testo 10.4-34.6 nmol/l and for LH 0.7-6.7 U/l).
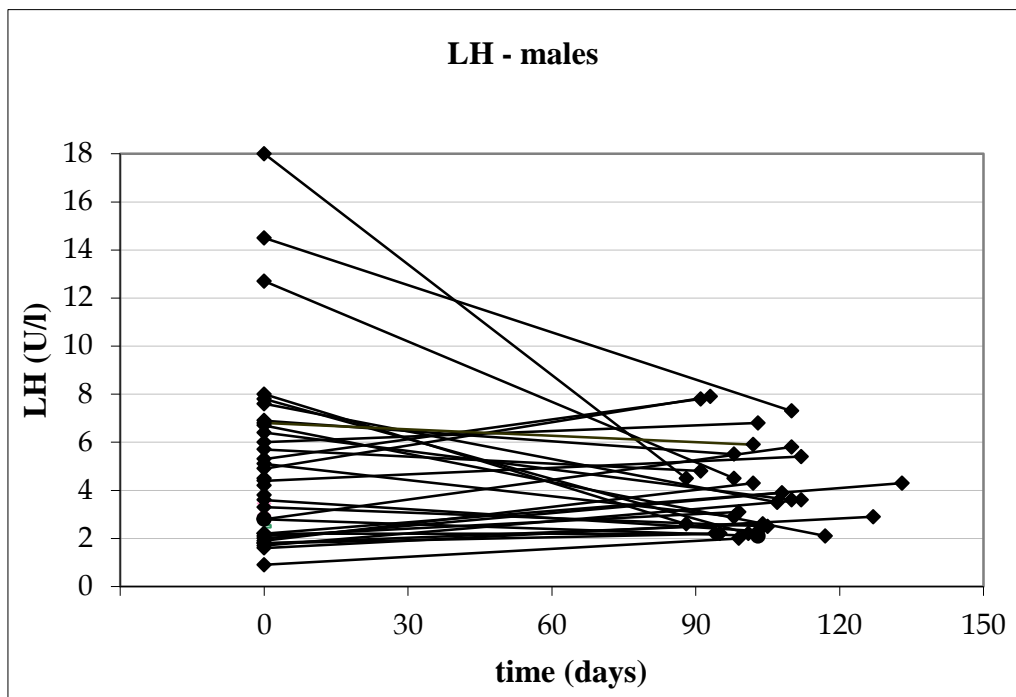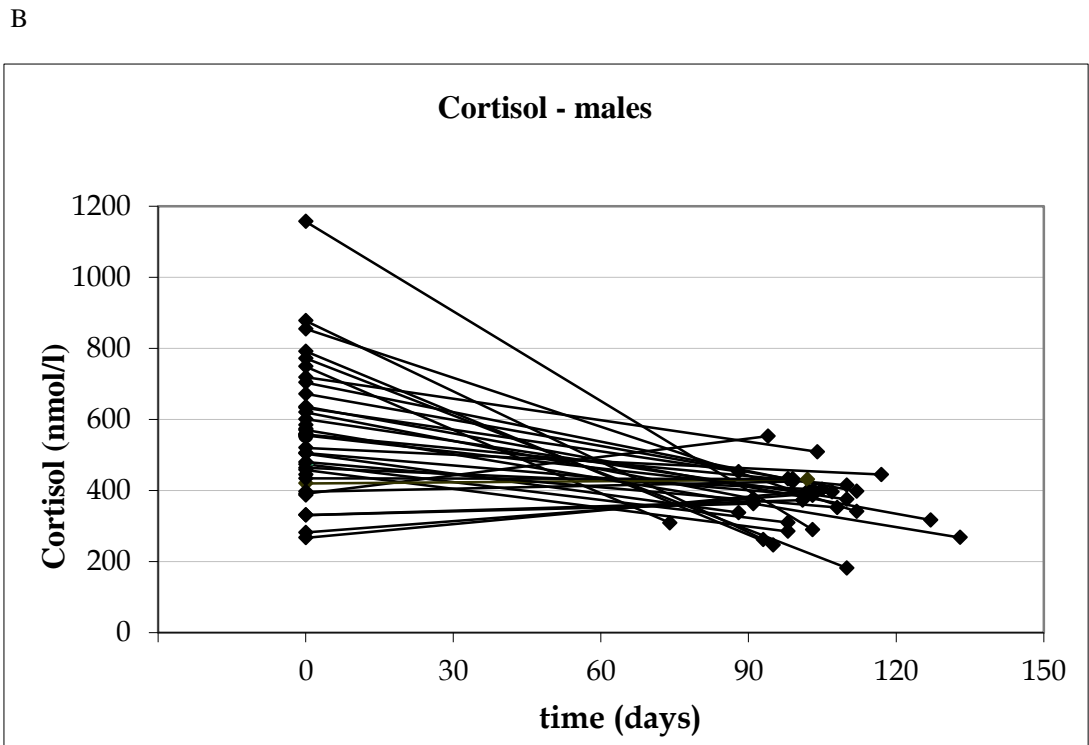
A
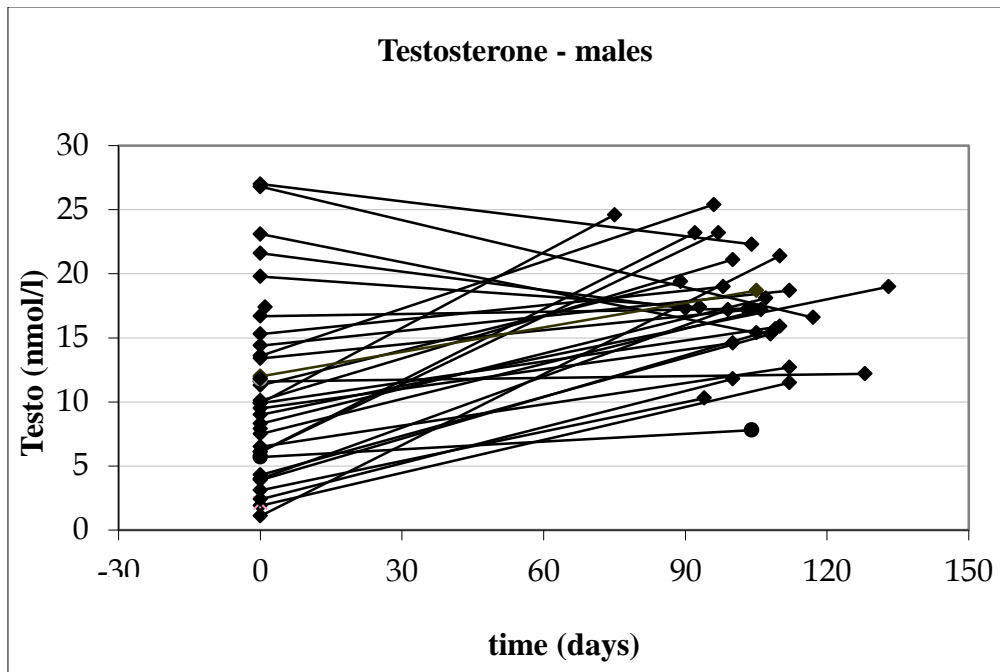
B

Figure 6. Serum cortisol levels in female (A) and male patients (B) during acute NE and after three months. The levels were higher during acute NE than 3 months after in both sexes. (*P*=0.004 for females and *P*<0.001 for males; reference range 180-680 nmol/l).
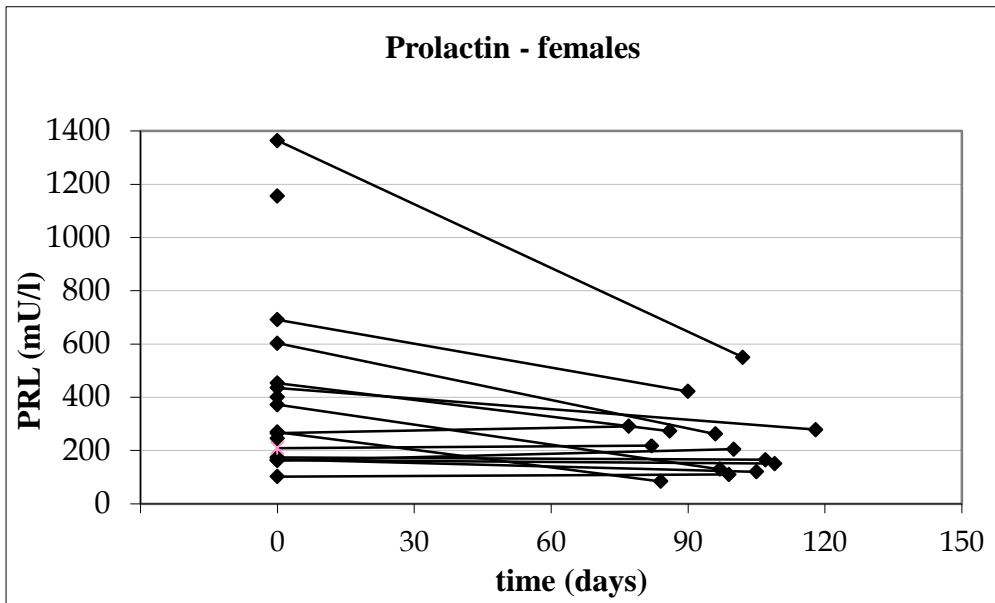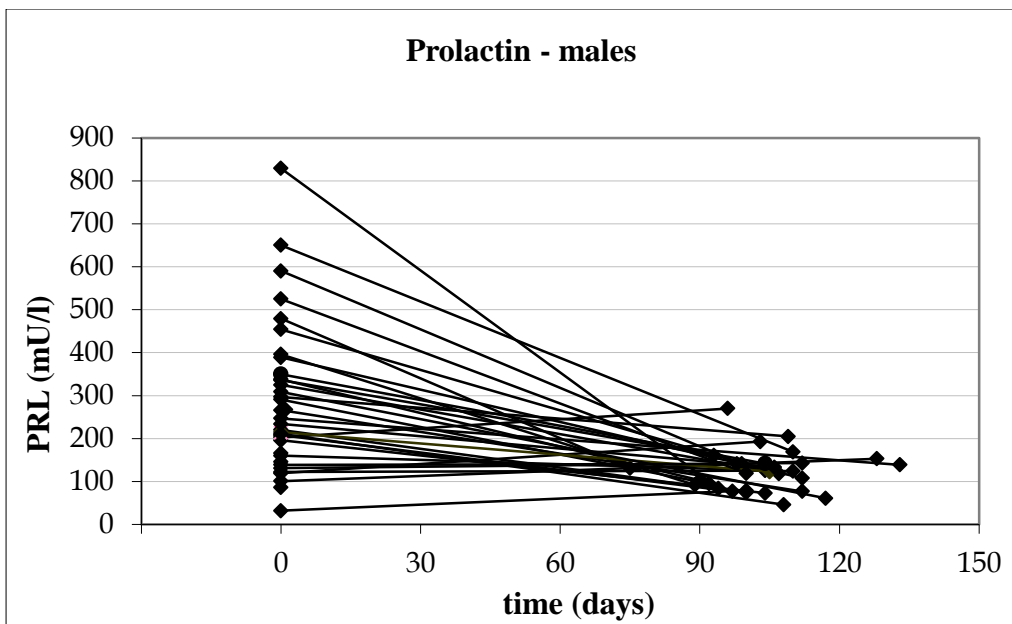
A

B
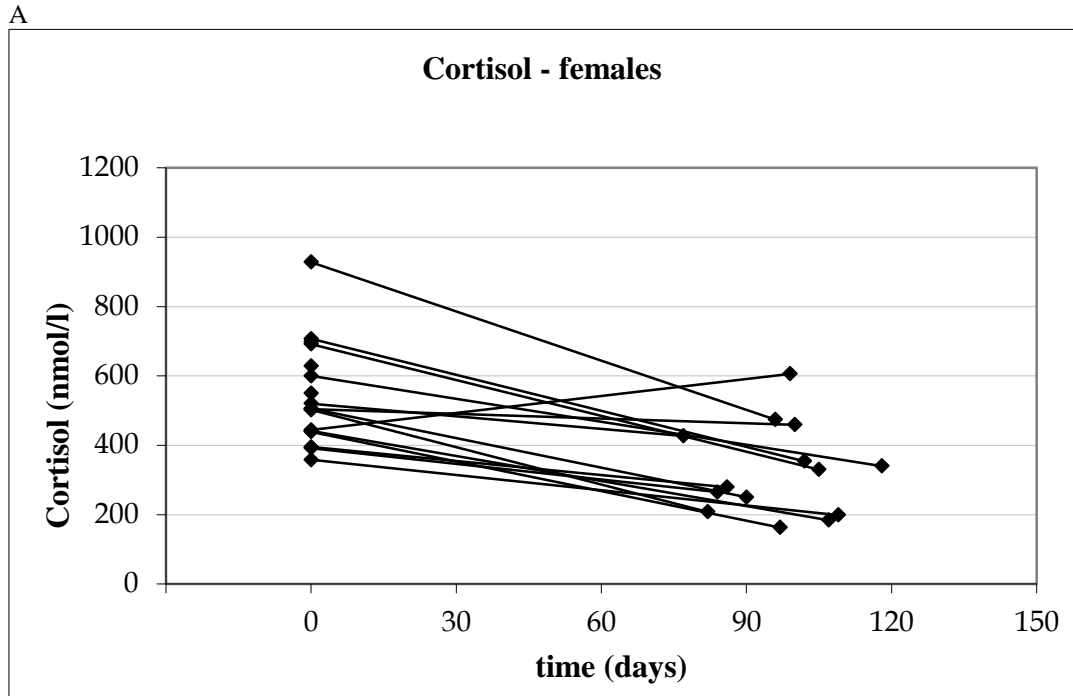
Figure 7. Serum prolactin levels in female (A) and male patients (B) during acute NE and after three months. The levels were higher during acute NE than 3 months after in both sexes. (*P*=0.017 for females and *P*<0.001 for males; reference values <600 mU/l in premenopausal women, <280 mU/L in postmenopausal women, and <450 mU/l in males).

A

Prolactin - females

B

Prolactin - males

5.3.2 Hormonal changes 1 to 10 years after acute NE

The serum levels of cortisol, PRL, fT4, TSH, Testo, LH, E2 and FSH measured 3 months after acute the NE did not differ from the levels measured at 1 or 5 years (data not shown). During the median follow-up of 5 years, 9 out of 54 (17 %) patients presented with chronic, overt hormonal deficits. Four out of these 9 patients presented with central hypogonadism (all males), 4 with primary hypothyroidism (3 males and 1 female), and one female with central hypogonadism and primary hypothyroidism. Due to the hormonal results, six out of these 9 patients were invited to an additional visit in 2008. A 43-year-old male patient with low testosterone and declining fT4 levels within the low reference range during acute NE, as well as at 3 and 12 months, died for an unknown reason soon after his 1-year follow-up visit. The abnormal hormonal levels suggesting central hypogonadism and possible hypothyroidism were analyzed post mortem. The overt hormonal deficiencies in the other patients were also noted only during the additional visit in 2008. Only one female patient was on L-thyroxine substitution which was started 9 years after acute NE. Neither age or gender distributions, nor the clinical severity of acute NE differed between patients with chronic hormonal defects and those with normal hormonal levels. The host genetic factors studied were not related to the prevalence of chronic hormone deficiencies (data not shown).

5.3.3 Radiological examinations

Thyroid ultrasound was performed on 5 patients with primary hypothyroidism during the additional visit in 2008, and showed diffuse or patchy hypoechogenicity compatible with thyroiditis in all cases. Only two of these five patients had measurable levels of TPOAb. A pituitary MRI scan was also undertaken in 2008 on

two male patients with symptoms and hormonal changes suggesting defects of central origin, one with apparent GH deficiency and one with secondary hypogonadism and probable secondary hypothyroidism. The MRI scans revealed a normal hypothalamic-pituitary region in both of them.

5.4 Glomerulonephritis diagnosed shortly after acute NE (Study V)

Study V describes seven patients with glomerulonephritis diagnosed shortly after acute NE. The acute phase of NE was clinically typical and serologically verified in all patients. The lowest blood platelet count varied from 32 to 159 x10^9/l, and the highest serum creatinine level ranged from 50 to 777 µmol/l and highest plasma CRP from 8 to 149 mg/l.

These patients evinced symptoms and signs of nephrotic syndrome and were readmitted to the hospital 1 to 12 weeks after acute NE. Table 9 shows the histological and laboratory data and treatment during the follow-up, and also the time to remission. Renal biopsies, revealing MPGN type 1 in 5 patients, and MGN and MesGn in one each, were performed 4 to 56 weeks after NE. The lowest serum albumin at the time of renal biopsy varied from 13 to 32 g/l, and patients with MPGN or mesangial GN (MesGN) also had microscopic hematuria. In addition, 5 of the patients were hypertensive.

The renal biopsies of the patients with MPGN were performed 4 to 5 weeks after acute NE (Table 9). They showed typical signs of MPGN type 1 with lobular appearance of the glomerular tuft, and mesangial expansion as well as hypercellularity. Also double contouring of the glomerular basement membrane was detected. However, infiltrating polymorphonuclear leukocytes in glomeruli (patient

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1), slight mononuclear cell infiltration in the interstitium (patient 2) and interstitial edema (patients 4 and 5) were also found in the renal biopsy specimens. IF results were available for patients 2-5, showing capillary IgG in patients 2, 3 and 5, IgM (patients 2-4) and C3 (patients 2-5) as well as C1q (patient 3), and also mesangial IgG and IgM (patient 2) and mesangial C3 (patients 2 and 5). Table 9 presents the follow-up characteristics of MPGN patients, showing the median time to remission to be 0.6 years. Two out of the 5 MPGN patients were treated with corticosteroids by reason of worsening renal function. Furthermore, two patients received anticoagulation therapy, one for severe nephrotic syndrome and the other for pulmonary embolization.

The renal biopsy specimen from patient 6 showed MGN with spikes and vacuoles on the capillary basement membrane without hypercellularity, and capillary IgG and C1q. In a biopsy of the patient with MesGn there was an increased mesangial matrix with mesangial IgG, IgM, IgA and C1q in IF study. The renal biopsy of the patient with MGN was performed over one year after the first symptoms and signs of nephrotic syndrome, as he was on anticoagulation therapy for thromboembolic complications. He received corticosteroids and cyclophosphamide for persistent severe nephrotic syndrome (Table 9). Table 9 shows the follow-up details of the MGN and MesGn patients, respectively.

As shown in Table 9, all patients achieved remission, the median time being 0.6 (range 0.5-5.5) years. The median follow-up time was 1.7 (0.7-15.6) years.

Table 9. Histological and laboratory findings, and time to remission in seven patients with glomerulonephritis shortly after acute NE.

Patient	Renal biopsy finding	Time from acute NE to renal biopsy (weeks)	Treatment	S-Crea at the time of renal biopsy (µmol/l)	S-Crea at the end of follow-up (µmol/l)	Proteinuria at the time of renal biopsy (g/d)	Proteinuria at the end of follow-up (g/d)	Time to remission (years)
1	MPGN	4	Di	200	79	14.9	< 0.30	1.3
2	MPGN	5	ACEi	91	80	7.3	0.24	0.5
3	MPGN	5	Di	62	49	9.8	0.17	0.6
4	MPGN	4	Di,Wa,Co	233	75	27.2	0.09	0.6
5	MPGN	5	Di,Wa,Co	186	114	4.3	0.15	0.5
6	MGN	56	ACEi,Di,Co,CP,Wa	90	80	21.2	0.30	5.5
7	MesGN	20	ARB	108	86	11.0	0.30	2.1

Abbreviations: MPGN=membranoproliferative glomerulonephritis, MGN=membranous glomerulonephritis, MesGN=mesangial glomerulonephritis, Di=diuretics, ACEi=angiotensin-converting enzyme inhibitor, Wa=warfarin, Co=corticosteroids, CP=cyclophosphamide, ARB=angiotensin receptor blocker, S-Crea=serum creatinine.

6. DISCUSSION

6.1   Long-term outcome of NE

The long-term prognosis of PUU-hantavirus-induced acute tubulointerstitial nephritis is favorable. Five to 6 years after acute NE patients here had higher GFR and urinary protein excretion, as well as higher 24-hour systolic BP compared to healthy controls, but the differences between the groups disappeared during the follow-up of 10 years. The possibility of an increased risk of elevated BP after acute NE nonetheless remains.

Limitations of studies I-III are relatively small sample sizes and the selection of only hospitalized NE patients, excluding milder forms of disease. Furthermore, study III was retrospective, and there were dropouts of 10 patients and 9 controls between the follow-ups from 5 to 10 years after acute NE. However, the results at 5 years were essentially the same whether these lost study subjects were included or not.

6.1.1   Renal function and proteinuria 6 and 10 years after acute NE

Study I showed that 6 years after acute NE patients had higher GFR than controls, and glomerular hyperfiltration was detected in 27 % of the patients. Furthermore, patients had more proteinuria and more often urinary excretion of α1-microglobulin than controls. Most previous publications on the long-term prognosis of renal function and proteinuria after NE or other hantavirus infections have been reports on

small groups of patients (Rubini et al. 1960, Lähdevirta 1971, Lähdevirta et al. 1978, Elisaf et al. 1993, Ala-Houhala et al. 2002) or seroepidemiological studies (Glass et al. 1990, Glass et al. 1993). Findings have suggested renal tubular dysfunction, proteinuria and decreased or increased creatinine clearance one to several years after acute hantavirus infection. In a recent retrospective study from Bosnia and Herzegovina, there were 14 patients with PUUV infection and 31 with DOBV infection, examined 10 years after acute disease (Tulumovic et al. 2010). The authors found that creatinine clearance in the DOBV-infected patients was lower than that in PUUV-infected patients, but was in both groups within normal limits.

The study by Mäkelä and co-workers (2000) and study I are the first reports involving increased GFR 5 to 6 years after AIN compared with healthy controls. In a study from Croatia, one third of HFRS patients likewise presented with increased creatinine clearance values from 3 to 6 years after acute disease (Ledina et al. 2003). Glomerular hyperfiltration is a common observation in early diabetes mellitus, and is closely related to the progression of diabetic nephropathy (Wirta and Pasternack 1995). Furthermore, there is evidence of increased GFR in early essential hypertension (Schmieder et al. 1997), and glomerular hyperfiltration predicting the development of microalbuminuria in hypertension (Palatini et al. 2006). Increased glomerular filtration rates have also been detected in early polycystic kidney disease (Wong et al. 2004). Probably the mechanisms causing hyperfiltration in these heterogeneous clinical situations are at least partly different.

In study I, the finding that patients were older than controls cannot explain the difference in GFR between the groups, since GFR usually declines with age. Patients also had higher fasting blood glucose levels and more often IFG than

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controls, which may have contributed to the results. However, there was no correlation between GFR and blood glucose, and GFR in patients with IFG or diabetes was essentially on the same level as GFR in those with normal blood glucose levels. Furthermore, in a previous study of the 5-year prognosis of NE with a similar difference in GFR between patient and control groups, there was no difference in fasting blood glucose levels (Mäkelä et al. 2000). Higher BP in patients compared with controls could also explain the difference in GFR, but there was no correlation between these parameters in study I. In addition, the increase in GFR was not accompanied by an increase in renal plasma flow, and the FF was thus higher in patients than controls. Mäkelä and co-workers (2000) suggest that this might contribute to increased intraglomerular pressure and GFR via an altered balance between the tone of afferent and efferent glomerular arterioles. However, the mechanism underlying the increased glomerular filtration rate 5 to 6 years after acute NE is not known, and according to study II neither the clinical severity of the acute phase of NE nor host genetic factors bore any relation to it.

Study III showed the long-term prognosis of NE to be favorable. The differences in renal function between the patient and controls groups 5 years after NE disappeared during the follow-up of 8 to 13 years. The GFR in patients decreased to the same level as controls. The BMI of the patients increased during the follow-up and at 10 years was significantly higher than that of the controls. There are some data indicating higher GFR in patients with overweight compared with lean subjects irrespective of the presence of hypertension (Ribstein et al. 1995). Although the patients in study III had gained weight, their GFR showed a decreasing trend. Longer follow-up would probably have shown whether the GFR in the patients is an

early sign of renal injury which could lead to a further decline in GFR compared with controls.

The urinary excretion of protein 6 years after NE was only mildly elevated in patients in study I, but nevertheless higher than in controls. The pathophysiological background to this finding is obscure, although renal biopsies from patients from 4 to 5 years after acute NE have shown increased numbers of hyalinized glomeruli, a slight thickening of basement membrane and periglomerular fibrosis as well as patchy interstitial fibrosis, tubular atrophy and tubular casts (Lähdevirta et al. 1978). In addition, Elisaf and co-workers (1993) carried out a renal biopsy on one patient one year after acute HFRS, and the specimen showed some degree of interstitial fibrosis and tubular atrophy together with sclerosed glomeruli. Further, higher BP and GFR in patients than in controls may explain the difference in proteinuria between the groups. Indeed, GFR correlated positively with urinary protein excretion in the patient group, whereas BP did not, which is in accord with previous findings (Mäkelä et al. 2000). According to study II, neither the clinical severity of acute NE nor host genetic factors explained the presence of proteinuria 6 years after acute NE. In study III, the difference between the patient and control groups in urinary excretion of protein no longer prevailed, confirming the favorable long-term renal outcome of NE.

Increased urinary excretion of α1-microglobulin was more usual in patients than in controls 6 years after NE in study I, as was also found in the earlier study by Mäkelä and co-workers (2000). Alpha-1-microglobulin is a low-molecular-weight glycoprotein produced by the liver and found in most organs, also in the kidney (Berggård et al. 1998). It is freely filtered by glomeruli, re-absorbed in proximal tubules and mostly degraded in the kidney, but a small part of it is also excreted in

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the urine. In one recent study it was concluded that the excretion rate of α1-microglobulin is higher in the daytime than during the night, and it is also higher in males than in females (Andersson et al. 2008). In studies I-III here, the urinary excretion of α1-microglobulin was measured only at nighttime. In studies I and II, 8 out of 9 patients showing increased excretion of α1-microglobulin were males. The biological function of α1-microglobulin is unknown, but it is considered to have immunosuppressive and radical reductant properties (Åkerstrom et al. 2007).

In renal diseases, increased urinary excretion of α1-microglobulin has been found to be a sign of disturbed tubular function and tubulointerstitial damage (Bazzi et al. 2000, Bakoush et al. 2001). Furthermore, some data suggest a role for α1-microglobulin in predicting an unfavorable outcome of acute tubular necrosis (Herget-Rosenthal et al. 2004, Parikh et al. 2010) and of glomerulopathies (Bazzi et al. 2001, Branten et al. 2005). There is also evidence from a study of drug-induced AIN that α1-microglobulin may predict the severity of tubular atrophy, interstitial edema and inflammatory infiltration in renal biopsy specimens (Wu et al. 2010). In studies I and II, the increased urinary excretion of α1-microglobulin in NE patients 6 years after acute phase can be considered to be a sign of tubular dysfunction.

In study II, neither the clinical severity of acute NE nor the amount of α1-microglobulin excretion during acute NE predicted the occurrence of α1-microglobulin excretion 6 years after. However, the highest plasma IL-6 levels during acute NE were higher in patients with increased urinary excretion of α1-micorglobulin 6 years later than in patients with no raised excretion of α1-microglobulin. The possible link between plasma IL-6 levels and increased excretion of α1-microglobulin is obscure. From a rat model there is controversial evidence of IL-6 stimulating the synthesis of α1-microglobulin in the liver

(Pierzchalski et al. 1992). Furthermore, in a recent study of newly diagnosed hypertensive patients, α1-microglobulin was found to be associated with circulating acute-phase proteins such as CRP, amyloid A and fibrinogen (Vyssoulis et al. 2007). The authors suggested that there can be increased synthesis of α1-microglobulin in the liver stimulated by proinflammatory cytokines, or increased urinary excretion of α1-microglobulin may also be a sign of disturbed proximal tubular function.

There are only a limited number of studies of the long-term outcome of AIN, and they mostly concern the drug-induced disease. Baker and Pusey (2004) published a review of 3 series of studies of AIN with different etiologies, of which about 15 % were infection-related. In the series in question, 12.5 % of the patients had remained on renal replacement therapy and 23.4 % had achieved partial recovery. Increasing age of the patients was associated with poorer prognosis, but not the peak serum creatinine concentration. In a retrospective study of 56 biopsied patients with AIN, median serum creatinine one year after acute disease was 140 µmol/l, whereafter most patients maintained a stable GFR (Clarkson et al. 2004). There are also conflicting findings regarding the correlation between renal biopsy findings of cellular infiltration, tubulitis, interstitial fibrosis in AIN and renal outcome (Baker and Pusey 2004, Praga and Gonzalez 2010). These observations may be due to the patchy nature of the disease and random sampling on renal biopsy (Baker and Pusey 2004).

In summary, the prospective study I confirmed the previous findings in a retrospective study that NE patients have a higher glomerular filtration rate and more daily urinary excretion of protein as well as overnight excretion of α1-microglobulin 6 years after the acute phase compared with healthy controls. Study II showed that these findings are not determined by the clinical severity of acute NE.

Finally, 10 years after acute NE the differences in GFR and proteinuria were no longer prevalent, confirming the favorable renal prognosis of NE (study III).

6.1.2 Blood pressure 6 and 10 years after acute NE

In study I, the mean and night-time systolic and night-time diastolic BP in ABP were higher in patients than in healthy controls 6 years after acute NE. This is in line with previous findings on BP in patients compared with controls 5 years after the acute phase (Mäkelä et al. 2000). Furthermore, in study I antihypertensive medication had been initiated in 5 out of 37 (14 %) patients after acute NE compared with none among the controls. Previous publications regarding the possible contribution of hantavirus infection to hypertension have been case reports with shorter follow-up times (Rubini et al. 1960, Lähdevirta 1971, Lähdevirta et al. 1978) or seroepidemiological studies (Glass et al. 1990, Glass et al. 1993, Niklasson et al. 1994). In one recent study of 10-year outcomes of PUUV- and DOBV-infected patients, there were altogether 42 % hypertensive patients and no difference between the groups in the prevalence of hypertension (Tulumovic et al. 2010). This finding, together with the results of study II, suggests that even mild-type hantavirus infection may predispose to hypertension. However, in the study from Bosnia and Herzegovina the possibility of elevated BP before PUUV or DOBV infection was not ruled out (Tulumovic et al. 2010). There are no data on BP after AIN with different etiologies.

The patients here were approximately 5 years older than the controls in study I. This may have contributed to the difference in BP between the groups, although there was no significant correlation between age and BP, and the difference between the groups also remained after adjusting for age. The results also remained the same

if subjects using antihypertensive medication were excluded from the study. Study II showed that neither the clinical severity of acute NE nor host genetic factors predict the emergence of elevated BP in patients 6 years after acute phase.

Study III showed that there was no difference in ABP between patients and controls 10 years after acute NE. Combined analysis defining hypertensive subjects as those using antihypertensive medication, having elevated BP in ABP or elevated office BP showed that 5 years after NE there were more hypertensive subjects among the patients compared to the control group. At 10 years 39 % of patients and 17 % of controls were hypertensive, but this difference was not statistically significant. On the other hand, at 10 years BMI in the patient group was higher compared with that of controls, this probably also contributing to the BP results. Furthermore, there were dropouts of 10 patients and 9 controls during the follow-up from 5 to 10 years, which may also have had an effect on the results, although the outcome of BP at 5 years was essentially the same with the larger group of patients and controls in the previous study (Mäkelä et al. 2000).

The possible etiopathogenetic link between hantavirus infection and hypertension is not known. It has been suggested that in the pathogenesis of hypertension renal involvement has an important role (Johnson et al. 2008). Hypertension may be initiated by the agents causing systemic and renal vasoconstriction, leading to intrarenal microvascular disease especially in the outer medulla (Johnson et al. 2002, Johnson et al. 2008). In theory, hantavirus infection might act as an initiating factor for renal vasoconstriction. Autopsy studies or renal biopsies from patients with hantavirus infection have shown that the renal medulla is the most seriously affected part of the kidney, with congestion and hemorrhages around the vessels together with interstitial inflammatory cell infiltrates (Lähdevirta 1971, Gajdusek

1982, Collan et al. 1991, Mustonen et al. 1994b). Moreover, the tendency of hantavirus to infect vascular endothelial cells could contribute to vascular damage (Yanagihara and Silverman 1990, Pensiero et al. 1992, Glass et al. 1993).

In summary, study I showed that patients have higher 24-hour systolic BP compared to the control group 6 years after acute NE, and that the severity of acute NE bears no relation to it, as shown in study II. However, 10 years after acute NE the difference in BP between the patient and control groups no longer persists, confirming the favorable outcome of the patients (study III). There was nevertheless a tendency to a greater prevalence of hypertension in the patient than control group, suggesting a possible role of hantavirus infection predisposing to hypertension.

6.2   Hormonal deficiencies and NE

The prevalence of hormonal abnormalities during the acute phase of NE has not hitherto been systematically evaluated. In study IV, 56 % of the patients with acute NE were found to have hypogonadism and/or hypothyroidism of either central or peripheral etiology.

Most hormonal deficiencies of the gonadal axis during the acute phase of NE were of central origin, and disappeared within 3 months. Approximately half of the male patients presented with low serum Testo levels and half of the premenopausal women with low serum E2 and FSH levels in the acute phase. The serum Testo levels in males and FSH levels in females increased significantly, while the serum LH levels of males did not change within 3 months, implying a central origin of temporary hypogonadism in most cases. However, there were also 8 male patients presenting with primary hypogonadism during acute NE, although clinical orchitis was not detected, and later 3 of them had chronically elevated serum LH levels with

serum Testo levels within normal limits. Altogether, the prevalence of primary hypogonadism in study IV with males of a median age of 39 years was higher than reported in males of this age group in general (Härkönen et al. 2003, Tajar et al. 2010).

Viral orchitis has been connected with several viruses, but is most commonly caused by the mumps virus, which may predispose to testicular failure, including decreased Testo production (Dejucq and Jegou 2001). Acute renal failure may also contribute to both central and peripheral hypogonadism, as has been suggested in previous studies (Kokot et al 1982, Levitan et al. 1984). The low serum Testo levels in male patients during the acute NE were related to the degree of inflammation and severity of acute renal failure. In one earlier study of male patients in an intensive care unit, the serum Testo and FSH levels were also lower in patients with severe illness compared with those with mild or moderate illness (Spratt et al. 1993). Many forms of stressors, for example infection, may suppress the activity of circulating gonadotropins and gonadal steroid hormones (Low 2007), which is in accord with the results of study IV. Furthermore, the high serum PRL levels detected during the acute phase of NE may have contributed to the high prevalence of central hypogonadism, as hyperprolactinemia is known to disrupt the central regulation of the gonadal axis (Bachelot and Binart 2007).

The present study (IV) also showed that serum cortisol, PRL and TSH levels were higher during the acute phase of NE compared with the results 3 months later. Furthermore, high serum cortisol and PRL levels during the acute phase were related to the severity of the disease. The fact that the kidneys are involved in the elimination of cortisol, PRL and TSH (Bauer et al. 1980, Whitworth et al. 1989, Kaptein 1996) may also have contributed to the increased serum levels, as the

clearance of these hormones may be diminished during acute renal failure. However, stress caused by acute illness is known to activate the hypothalamic-pituitary-adrenal axis, and cytokines such as IL-1β have also been suggested to have the same effect (Low 2007).

Altogether 17 % of the present cohort of NE patients developed chronic hormonal deficiencies during a median follow-up of five years. Four males and one female among these 9 patients presented with central hypogonadism. In a study by Stojanovic and co-workers (2009) involving 60 patients with previous HFRS, 18 % had at least one pituitary hormone deficit. Four out of five patients with multiple pituitary hormone deficiencies were further evaluated by brain MRI, which revealed empty sella in all of them. In the present series (IV) the brain MRI scan yielded no pathological findings in the patients studied for central hormonal defects. Previous case reports and autopsy studies have reported pituitary hemorrhage complicating acute hantavirus infection (Suh et al. 1995, Valtonen et al. 1995, Hautala et al. 2002), which may lead to central hormonal deficiencies. The mechanisms of pituitary hemorrhage are obscure, but hypotension or low platelet count may contribute to it. Also vascular injury and dysfunction of platelets induced by uremia may predispose to a risk of bleeding (Cosgriff 1991). In study IV here neither platelet count nor hypotension during the acute phase of NE correlated with hormonal changes during the acute phase or later.

It is possible that mechanisms other than ischemia or hemorrhage of the pituitary gland contribute to the development of the hormonal dysfunctions, since there are reports of pituitary hormone deficits after NE (Settergren et al. 1992) or other hantavirus infections (Lim et al. 1986) without any signs of radiological pituitary abnormalities. PUUV antigen has been found in the pituitary gland of an NE patient

at autopsy (Hautala et al. 2002), but so far there is no evidence of a direct cytopathic effect of hantaviruses on pituitary cells. Probably the immune-mediated damage to the endothelial cells and increased vascular permeability in acute hantavirus infection also contribute to pituitary hormone abnormalities.

Peripheral hormonal disturbances were surprisingly common in the present material (IV), although neither clinical symptoms nor signs of thyroiditis or orchitis have so far been described in acute NE. The prevalence of hypothyroidism was 24 % in female patients and 8 % in males, figures clearly higher than the prevalences found in the general population (Bjoro et al. 2000, Hollowell et al. 2002). Thyroid ultrasound examination of the patients with primary hypothyroidism after acute NE showed a hypoechogenic structure of the thyroid gland, indicating chronic thyroiditis (Hegedüs 2001). Three out of 5 patients had no antibodies against thyroid peroxidase, which suggests a non-autoimmune etiology of their thyroiditis. The mechanisms underlying peripheral thyroidal or testicular hormonal alterations during or after acute NE are unclear. The role of immune-mediated endothelial cell damage induced by overproduction of cytokines during acute NE and contributing to the increased vascular permeability can be suspected. However, the peripheral hormonal disturbances did not correlate with the plasma levels of cytokines in study IV. According to one recent review, viral infections may contribute to Hashimoto's thyroiditis via bystander activation of autoreactive T cells or via signaling molecules associated with antiviral responses (Mori and Yoshida 2010).

The chronic hormonal defects detected in study IV did not correlate with the clinical severity of acute NE or with host genetic factors. In a retrospective study by Stojanovic and co-workers (2009), 6 months to 17 years after HFRS, GH, T_4 and Testo deficiencies were all related to the severity of acute disease. In that study the

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clinical picture of acute HFRS was more severe, as 58 % of the patients required dialysis treatment compared with 6 % in the present study. However, the Serbian paper did not report any peripheral hormonal defects (Stojanovic et al. 2009), and it is possible that the mechanisms behind central and peripheral hormonal abnormalities are different.

By reason of the retrospective nature of the study, there are no complete data on possible symptoms and signs of hormonal alterations during the acute phase or follow-up. This may have led to underestimation of the prevalence of hormonal sequelae, in that milder changes cannot be diagnosed by means of basal hormone levels alone. Interestingly, fatigue lasting several weeks after acute NE is usual (Lähdevirta 1971), and it has been suggested that in an infectious CNS disease this might be explained by central hormonal deficiencies (Schaefer et al. 2008).

In summary, hormonal abnormalities during acute NE were common (study IV), and they correlated with the severity of the acute disease, i.e., the levels of renal failure and inflammatory markers. A considerable proportion (17 %) of the patients also developed chronic hormonal deficiencies, which could not be predicted by the severity of acute NE, or by the host genetic factors studied.

6.3 Glomerulonephritis and NE

Study V reported on 7 patients with symptoms and signs of nephrotic syndrome emerging shortly after serologically verified acute NE. In 5 of these patients, MPGN was diagnosed by renal biopsy, and in 2 patients MGN and MesGN, respectively. All patients achieved remission in approximately 0.6 years.

Establishment of an etiopathogenetic connection between viral infection and glomerular disease requires the clinical symptoms of viral infection, serological

diagnosis, identification of specific viral antigenemia, and the detection of viral antigens and host antibodies in renal biopsy specimens (di Belgiojoso et al. 2002). Additional criteria are an improvement of renal disease with clearance of the suspected antigen or a recurrence of it after reinfection (di Belgiojoso et al. 2002). The glomerular injury in viral infections may be mediated by circulating immunocomplexes, which can be derived from viral antigens or host antiviral antibodies, or from endogenous antigens modified by viral injury and host auto-antibodies (Glassock 1991). Viral antigens binding to the glomerular structures may also cause injury by *in situ* immune-mediated mechanisms (Golbus and Wilson 1979, Couser 1985). In addition, expression of viral proteins or abnormal host proteins can induce cell dysfunction, necrosis (di Belgiojoso et al. 2002) or apoptosis (Conaldi et al. 1998), or increased matrix synthesis and decreased matrix degradation (di Belgiojoso et al. 2002), or release of chemokines (Segerer et al. 2000), cytokines, adhesion molecules and growth factors (di Belgiojoso et al. 2002). Furthermore, circulating cryoglobulins may be induced by the host response to viral infection (D'Amico 1998), and a direct cytopathic effect of virus on glomerular cells is also possible (Glassock et al. 1990). It is probable that different viruses induce renal injury by different mechanisms, but an unambiguous causal relation between viral infection and renal disease is usually difficult to establish (di Belgiojoso et al. 2002).

During recent years, more data have been obtained on the possible role of toll-like receptors (TLRs) in the pathogenesis of immune complex-mediated glomerulonephritis. TLRs are transmembrane proteins which can recognize pathogen-associated molecular patterns and also be activated by endogenous ligands. Based on mouse models it has been suggested that TLRs could mediate

glomerular injury by modulating the expression of chemokines and contributing to the deposition of immunocomplexes (Banas et al. 2008). TLRs have been linked to HCV–associated MPGN (Wörnle et al. 2006), and interestingly, in a recent in vitro study HTNV was shown to induce the expression of TLR4 and enhance the production of cytokines (Jiang et al. 2008).

The etiopathogenetic link between PUUV infection and glomerulonephritis is obscure. In the present context (V) and in a study by Mustonen and co-workers (2001), PUUV was not sought in renal biopsy specimens. A previous immunohistochemical study of renal biopsies from acute NE patients reported no evidence of PUUV in the biopsy specimens (Temonen et al. 1996). HTNV antigen has been reported to be found in the epithelial cells of the distal nephrons in one case of fatal disease (Poljak and Avsic Zupanc 1994). Groen and co-workers (1996) found PUUV antigen in 6 out of 10 renal biopsies from seropositive patients, localized in the cytoplasm of the tubular epithelial cells. Furthermore, in a study from Korea, 22 out of 23 renal biopsy specimens from HTNV patients showed viral glycoproteins in the cytoplasm of the tubular epithelial cells (Kim et al. 1993). In HCPS, hantavirus has been found in the endothelial cells of interstitial capillaries in the renal medulla (Zaki et al. 1995). There is no evidence of persistent or recurrent hantavirus infections. Viral RNA can be found within the first 9 days of symptomatic PUUV infection, but convalescent samples are PCR-negative (Hörling et al. 1995, Plyusnin et al. 1997b, Plyusnin et al. 1999).

The type of exudative MPGN has previously been linked to infectious diseases (D'Amico and Ferrario 1992). In the present series (V), a renal biopsy from one patient with MPGN showed infiltrating polymorphonuclear leukocytes in the glomeruli, giving the appearance of the exudative form of MPGN. Furthermore, in

the aforementioned study by Mustonen and co-workers (2001), 4 out of the 5 MPGN patients had exudative features detected in renal biopsies.

Usually the clinical feature of adult idiopathic MPGN varies from nephrotic-range proteinuria to nephrotic syndrome, hematuria, hypertension and some degree of renal function impairment (Nakopoulou 2001). MPGN is considered to carry an unfavorable prognosis, since 5 years after diagnosis 50 % of patients either need renal replacement therapy or die (Schmitt et al. 1990). Two patients in study V received corticosteroids, one with a clinical picture of rapidly progressive glomerulonephritis and the other also with worsening renal function. However, the impact of corticosteroids on the clinical course of MPGN in these patients remains uncertain. Hemodynamic changes may have explained the worsening renal function, as both diuretic therapy and nephrotic syndrome can predispose to relative hypovolemia and renal ischemia (Orth and Ritz 1998). The efficacy of various therapeutic regimens, including corticosteroids, in adult MPGN is difficult to assess in the absence of larger and controlled trials (Levin 1999). Spontaneous remission of MPGN is also rare, reported in only 2-20 % of cases (Williams 1998). However, Mustonen and co-workers (2001) report the incidence of remission of MPGN to be exceptionally high, implying a better prognosis of MPGN after acute NE than usual. Nevertheless, one patient in that study developed end-stage renal failure. In a recent case report of EBV infection –related MPGN, remission of the nephrotic syndrome was achieved within 6 months (Karamadoukis 2008). The clinical course of MPGN associated with HCV infection varies, and treatment with antiviral agents or anti-inflammatory or cytotoxic drugs has been tried with variable responses (Meyers et al. 2003).

Study V also reports on patients with MesGN and MGN shortly after acute NE. The patient with MesGN had heavy proteinuria during the time of renal biopsy but went into spontaneous remission within 2 years. The patient with MGN had severe and relapsing nephrotic syndrome with thromboembolic complications, and was treated with corticosteroids and cyclophosphamide. The treatment of MGN with corticosteroids or cytotoxic drugs is controversial by reason of the probability of persistent spontaneous remission of about 30 % in untreated patients with nephrotic syndrome (Ponticelli 2007). Choice of treatment thus requires a thorough evaluation of probable benefits and risks. MGN associated with HCV or HBV infection may be treated with antiviral drugs (Ponticelli 2007). In a recent case of an infant with CMV infection and MGN, ganciclovir therapy followed by valganciclovir was administered and remission achieved (Georgaki-Angelaki et al. 2009).

In summary, study V yielded additional data on the relationship of PUUV infection and subsequent glomerulonephritis. The prognosis of MPGN emerging shortly after acute NE seems to be favorable compared with its usual clinical course with nephrotic syndrome, although larger follow-up studies are called for to confirm this finding. Further, patients with MGN and MesGN shortly after acute NE were described.

7. SUMMARY AND CONCLUSIONS

The main findings in the present series were as follows:

1. NE patients had higher GFR, more proteinuria and higher BP than seronegative, healthy controls 5 to 6 years after the acute phase. However, after 10 years' follow-up, the differences between the patient and control group in renal function, proteinuria and BP disappeared, confirming the favorable long-term outcome of NE.

2. The clinical severity of acute NE had no association with renal function, the prevalence of proteinuria or the level of BP 6 years later.

3. Gonadal and thyroid hormone deficits were common during acute NE, and central deficiencies were associated with the severity of renal failure and inflammation. Surprisingly many patients developed chronic central and peripheral hormonal deficiencies, which were not related to the severity of acute NE. This suggests that even relatively mild NE may predispose patients to chronic hormonal alterations.

4. Glomerulonephritis may complicate the convalescent phase of acute NE. Renal biopsy specimens showed MPGN in five patients, and MGN and MesGN in one each. The median time to remission was 0.6 years, indicating a favorable outcome in most cases.

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REFERENCES

(1999): 1999 World Health Organization-International Society of Hypertension Guidelines for the Management of Hypertension. Guidelines Subcommittee. J Hypertens 17:151-183.

Ahlm C, Linderholm M, Juto P, Stegmayr B and Settergren B (1994a): Prevalence of serum IgG antibodies to Puumala virus (haemorrhagic fever with renal syndrome) in northern Sweden. Epidemiol Infect 113:129-136.

Ahlm C, Settergren B, Gothefors L and Juto P (1994b): Nephropathia epidemica (hemorrhagic fever with renal syndrome) in children: clinical characteristics. Pediatr Infect Dis J 13:45-49.

Ahlm C, Linden C, Linderholm M, Alexeyev OA, Billheden J, Elgh F, Fagerlund M, Zetterlund B and Settergren B (1998): Central nervous system and ophthalmic involvement in nephropathia epidemica (European type of haemorrhagic fever with renal syndrome). J Infect 36:149-155.

Ala-Houhala I, Makela S, Koivunen E, Mustonen J and Pasternack A (2000): Pronounced monocytosis in a case of nephropathia epidemica. Scand J Infect Dis 32:419-420.

Ala-Houhala I, Koskinen M, Ahola T, Harmoinen A, Kouri T, Laurila K, Mustonen J and Pasternack A (2002): Increased glomerular permeability in patients with nephropathia epidemica caused by Puumala hantavirus. Nephrol Dial Transplant 17:246-252.

Alexeyev OA and Morozov VG (1995): Neurological manifestations of hemorrhagic fever with renal syndrome caused by Puumala virus: review of 811 cases. Clin Infect Dis 20:255-258.

Alexeyev OA, Ahlm C, Billheden J, Settergren B, Wadell G and Juto P (1994): Elevated levels of total and Puumala virus-specific immunoglobulin E in the Scandinavian type of hemorrhagic fever with renal syndrome. Clin Diagn Lab Immunol 1:269-272.

Andersson L, Haraldsson B, Johansson C and Barregard L (2008): Methodological issues on the use of urinary alpha-1-microglobuline in epidemiological studies. Nephrol Dial Transplant 23:1252-1256.

Antoniadis A, Stylianakis A, Papa A, Alexiou-Daniel S, Lampropoulos A, Nichol ST, Peters CJ and Spiropoulou CF (1996): Direct genetic detection of Dobrava virus in Greek and Albanian patients with hemorrhagic fever with renal syndrome. J Infect Dis 174:407-410.

Armstrong LR, Zaki SR, Goldoft MJ, Todd RL, Khan AS, Khabbaz RF, Ksiazek TG and Peters CJ (1995): Hantavirus pulmonary syndrome associated with entering or cleaning rarely used, rodent-infested structures. J Infect Dis 172:1166.

Avsic-Zupanc T, Xiao SY, Stojanovic R, Gligic A, van der Groen G and LeDuc JW (1992): Characterization of Dobrava virus: a Hantavirus from Slovenia, Yugoslavia. J Med Virol 38:132-137.

Avsic-Zupanc T, Petrovec M, Furlan P, Kaps R, Elgh F and Lundkvist Å (1999): Hemorrhagic fever with renal syndrome in the Dolenjska region of Slovenia--a 10-year survey. Clin Infect Dis 28:860-865.

Avsic-Zupanc T, Nemirov K, Petrovec M, Trilar T, Poljak M, Vaheri A and Plyusnin A (2000): Genetic analysis of wild-type Dobrava hantavirus in Slovenia: co-existence of two distinct genetic lineages within the same natural focus. J Gen Virol 81:1747-1755.

Bachelot A and Binart N (2007): Reproductive role of prolactin. Reproduction 133:361-369.

Baker RJ and Pusey CD (2004): The changing profile of acute tubulointerstitial nephritis. Nephrol Dial Transplant 19:8-11.

Bakoush O, Grubb A, Rippe B and Tencer J (2001): Urine excretion of protein HC in proteinuric glomerular diseases correlates to urine IgG but not to albuminuria. Kidney Int 60:1904-1909.

Banas MC, Banas B, Hudkins KL, Wietecha TA, Iyoda M, Bock E, Hauser P, Pippin JW, Shankland SJ, Smith KD, Stoelcker B, Liu G, Gröne HJ, Krämer BK and Alpers CE

(2008): TLR4 links podocytes with the innate immune system to mediate glomerular injury. J Am Soc Nephrol 19:704-713.

Baskin HJ, Cobin RH, Duick DS, Gharib H, Guttler RB, Kaplan MM and Segal RL (2002): American Association of Clinical Endocrinologists medical guidelines for clinical practice for the evaluation and treatment of hyperthyroidism and hypothyroidism. Endocr Pract 8:457-469.

Bauer AG, Wilson JH and Lamberts SW (1980): The kidney is the main site of prolactin elimination in patients with liver disease. J Clin Endocrinol Metab 51:70-73.

Bazzi C, Petrini C, Rizza V, Arrigo G and D'Amico G (2000): A modern approach to selectivity of proteinuria and tubulointerstitial damage in nephrotic syndrome. Kidney Int 58:1732-1741.

Bazzi C, Petrini C, Rizza V, Arrigo G, Beltrame A, Pisano L and D'Amico G (2001): Urinary excretion of IgG and alpha(1)-microglobulin predicts clinical course better than extent of proteinuria in membranous nephropathy. Am J Kidney Dis 38:240-248.

Berggård T, Oury TD, Thøgersen IB, Åkerström B and Enghild JJ (1998): Alpha1-microglobulin is found both in blood and in most tissues. J Histochem Cytochem 46:887-894.

Bergmann F, Krone B, Bleich S, Prange H and Paulus W (2002): Encephalitis due to a hantavirus infection. J Infect 45:58-59.

Bharadwaj M, Nofchissey R, Goade D, Koster F and Hjelle B (2000): Humoral immune responses in the hantavirus cardiopulmonary syndrome. J Infect Dis 182:43-48.

Bi Z, Formenty PB and Roth CE (2008): Hantavirus infection: a review and global update. J Infect Dev Ctries 2:3-23.

Bjøro T, Holmen J, Krüger O, Midthjell K, Hunstad K, Schreiner T, Sandnes L and Brochmann H (2000): Prevalence of thyroid disease, thyroid dysfunction and thyroid peroxidase antibodies in a large, unselected population. The Health Study of Nord-Trondelag (HUNT). Eur J Endocrinol 143:639-647.

Borges AA, Campos GM, Moreli ML, Moro Souza RL, Saggioro FP, Figueiredo GG, Livonesi MC and Moraes Figueiredo LT (2008): Role of mixed Th1 and Th2 serum cytokines on pathogenesis and prognosis of hantavirus pulmonary syndrome. Microbes Infect 10:1150-1157.

Borges AA, Donadi EA, Campos GM, Moreli ML, de Sousa RL, Saggioro FP, de Figueiredo GG, Badra SJ, Deghaide NH and Figueiredo LT (2010): Association of - 308G/A polymorphism in the tumor necrosis factor-alpha gene promoter with susceptibility to development of hantavirus cardiopulmonary syndrome in the Ribeirao Preto region, Brazil. Arch Virol 155:971-975.

Bostik P, Winter J, Ksiazek TG, Rollin PE, Villinger F, Zaki SR, Peters CJ and Ansari AA (2000): Sin nombre virus (SNV) Ig isotype antibody response during acute and convalescent phases of hantavirus pulmonary syndrome. Emerg Infect Dis 6:184-187.

Branten AJ, du Buf-Vereijken PW, Klasen IS, Bosch FH, Feith GW, Hollander DA and Wetzels JF (2005): Urinary excretion of beta2-microglobulin and IgG predict prognosis in idiopathic membranous nephropathy: a validation study. J Am Soc Nephrol 16:169-174.

Braun N, Haap M, Overkamp D, Kimmel M, Alscher MD, Lehnert H and Haas CS (2010): Characterization and outcome following Puumala virus infection: a retrospective analysis of 75 cases. Nephrol Dial Transplant 25:2997-3003.

Bridson E (2001): The English 'sweate' (Sudor Anglicus) and Hantavirus pulmonary syndrome. Br J Biomed Sci 58:1-6.

Brummer-Korvenkontio M, Vaheri A, Hovi T, von Bonsdorff CH, Vuorimies J, Manni T, Penttinen K, Oker-Blom N and Lähdevirta J (1980): Nephropathia epidemica: detection of antigen in bank voles and serologic diagnosis of human infection. J Infect Dis 141:131-134.

Brummer-Korvenkontio M, Vapalahti O, Henttonen H, Koskela P, Kuusisto P and Vaheri A (1999): Epidemiological study of nephropathia epidemica in Finland 1989-96. Scand J Infect Dis 31:427-435.

116

Castillo C, Naranjo J, Sepulveda A, Ossa G and Levy H (2001): Hantavirus pulmonary syndrome due to Andes virus in Temuco, Chile: clinical experience with 16 adults. Chest 120:548-554.

Chen LB and Yang WS (1990): Abnormalities of T cell immunoregulation in hemorrhagic fever with renal syndrome. J Infect Dis 161:1016-1019.

Clarkson MR, Giblin L, O'Connell FP, O'Kelly P, Walshe JJ, Conlon P, O'Meara Y, Dormon A, Campbell E and Donohoe J (2004): Acute interstitial nephritis: clinical features and response to corticosteroid therapy. Nephrol Dial Transplant 19:2778-2783.

Clement J, Colson P and McKenna P (1994): Hantavirus pulmonary syndrome in New England and Europe. N Engl J Med 331:545-546; author reply 547-548.

Clement J, Mustonen J, Van Damme B, Helin H, Maes P and Van Ranst M (2011): Severe crescentic glomerulonephritis linked to an acute Hantaan virus infection? Nephrol Dial Transplant 26:1448-1449.

Collan Y, Lähdevirta J and Jokinen EJ (1978a): Electron Microscopy of Nephropathia Epidemica. Glomerular changes. Virchows Arch A Pathol Anat Histol 377:129-144.

Collan Y, Lähdevirta J and Jokinen EJ (1978b): Electron microscopy of nephropathia epidemica. Renal tubular basement membrane. Am J Pathol 92:167-172.

Collan Y, Mihatsch MJ, Lähdevirta J, Jokinen EJ, Romppanen T and Jantunen E (1991): Nephropathia epidemica: mild variant of hemorrhagic fever with renal syndrome. Kidney Int Suppl 35:S62-71.

Conaldi PG, Biancone L, Bottelli A, Wade-Evans A, Racusen LC, Boccellino M, Orlandi V, Serra C, Camussi G and Toniolo A (1998): HIV-1 kills renal tubular epithelial cells in vitro by triggering an apoptotic pathway involving caspase activation and Fas upregulation. J Clin Invest 102:2041-2049.

Cosgriff TM (1991): Mechanisms of disease in Hantavirus infection: pathophysiology of hemorrhagic fever with renal syndrome. Rev Infect Dis 13:97-107.

Couser WG (1985): Mechanisms of glomerular injury in immune-complex disease. Kidney Int 28:569-583.

Crespin L, Verhagen R, Stenseth NC, Yoccoz NG, Prevot-Julliard AC and Lebreton JD (2002): Survival in fluctuating bank vole populations: seasonal and yearly variations. OIKOS 98:467-479.

Crowcroft NS, Infuso A, Ilef D, Le Guenno B, Desenclos JC, Van Loock F and Clement J (1999): Risk factors for human hantavirus infection: Franco-Belgian collaborative case-control study during 1995-6 epidemic. Bmj 318:1737-1738.

D'Amico G (1998): Renal involvement in hepatitis C infection: cryoglobulinemic glomerulonephritis. Kidney Int 54:650-671.

D'Amico G and Ferrario F (1992): Mesangiocapillary glomerulonephritis. J Am Soc Nephrol 2:S159-166.

Davis IC, Zajac AJ, Nolte KB, Botten J, Hjelle B and Matalon S (2002): Elevated generation of reactive oxygen/nitrogen species in hantavirus cardiopulmonary syndrome. J Virol 76:8347-8359.

de Carvalho Nicacio C, Björling E and Lundkvist Å (2000): Immunoglobulin A responses to Puumala hantavirus. J Gen Virol 81:1453-1461.

Dejucq N and Jegou B (2001): Viruses in the mammalian male genital tract and their effects on the reproductive system. Microbiol Mol Biol Rev 65:208-231

Desailloud R and Hober D (2009): Viruses and thyroiditis: an update. Virol J 6:5.

di Belgiojoso GB, Ferrario F and Landriani N (2002): Virus-related glomerular diseases: histological and clinical aspects. J Nephrol 15:469-479.

Dietl CA, Wernly JA, Pett SB, Yassin SF, Sterling JP, Dragan R, Milligan K and Crowley MR (2008): Extracorporeal membrane oxygenation support improves survival of patients with severe Hantavirus cardiopulmonary syndrome. J Thorac Cardiovasc Surg 135:579-584.

Duchin JS, Koster FT, Peters CJ, Simpson GL, Tempest B, Zaki SR, Ksiazek TG, Rollin PE, Nichol S, Umland ET et al. (1994): Hantavirus pulmonary syndrome: a clinical description of 17 patients with a newly recognized disease. The Hantavirus Study Group. N Engl J Med 330:949-955.

118

Dunst R, Mettang T and Kuhlmann U (1998): Severe thrombocytopenia and response to corticosteroids in a case of nephropathia epidemica. Am J Kidney Dis 31:116-120.

Elisaf M, Korakis H and Siamopoulos KC (1993): Chronic renal dysfunction in hemorrhagic fever with renal syndrome patients. Ren Fail 15:623-627.

Ennis FA, Cruz J, Spiropoulou CF, Waite D, Peters CJ, Nichol ST, Kariwa H and Koster FT (1997): Hantavirus pulmonary syndrome: CD8+ and CD4+ cytotoxic T lymphocytes to epitopes on Sin Nombre virus nucleocapsid protein isolated during acute illness. Virology 238:380-390.

Esselink RA, Gerding MN, Brouwers PJ, Solleveld H, Jordans JG, Groen J and Osterhaus AD (1994): Guillain-Barre syndrome associated with hantavirus infection. Lancet 343:180-181.

Evander M and Ahlm C (2009): Milder winters in northern Scandinavia may contribute to larger outbreaks of haemorrhagic fever virus. Glob Health Action 2:

Evander M, Eriksson I, Pettersson L, Juto P, Ahlm C, Olsson GE, Bucht G and Allard A (2007): Puumala hantavirus viremia diagnosed by real-time reverse transcriptase PCR using samples from patients with hemorrhagic fever and renal syndrome. J Clin Microbiol 45:2491-2497.

Forouhi NG, Balkau B, Borch-Johnsen K, Dekker J, Glumer C, Qiao Q, Spijkerman A, Stolk R, Tabac A and Wareham NJ (2006): The threshold for diagnosing impaired fasting glucose: a position statement by the European Diabetes Epidemiology Group. Diabetologia 49:822-827.

Forsius A (1995): Myyräkuumeen historiaa. Suomen Lääkärilehti 16:1921.

Forslund T, Saltevo J, Anttinen J, Auvinen S, Brummer-Korvenkontio M, Korhonen A and Poutiainen M (1992): Complications of nephropathia epidemica: three cases. J Intern Med 232:87-90.

Gajdusek DC (1962): Virus hemorrhagic fevers. Special reference to hemorrhagic fever with renal syndrome (epidemic hemorrhagic fever). J Pediatr 60:841-857.

Gajdusek DC (1982): Muroid virus nephropathies and muroid viruses of the Hantaan virus group. Scand J Infect Dis Suppl 36:96-108.

Gavrilovskaya IN, Shepley M, Shaw R, Ginsberg MH and Mackow ER (1998): beta3 Integrins mediate the cellular entry of hantaviruses that cause respiratory failure. Proc Natl Acad Sci U S A 95:7074-7079.

Gavrilovskaya IN, Brown EJ, Ginsberg MH and Mackow ER (1999): Cellular entry of hantaviruses which cause hemorrhagic fever with renal syndrome is mediated by beta3 integrins. J Virol 73:3951-3959.

Gavrilovskaya IN, Gorbunova EE, Mackow NA and Mackow ER (2008): Hantaviruses direct endothelial cell permeability by sensitizing cells to the vascular permeability factor VEGF, while angiopoietin 1 and sphingosine 1-phosphate inhibit hantavirus-directed permeability. J Virol 82:5797-5806.

Georgaki-Angelaki H, Lycopoulou L, Stergiou N, Lazopoulou D, Paraskevakou H, Giannaki-Psinaki M and Mentis A (2009): Membranous nephritis associated with acquired cytomegalovirus infection in a 19-month-old baby. Pediatr Nephrol 24:203-206.

Glass GE, Childs JE, Watson AJ and LeDuc JW (1990): Association of chronic renal disease, hypertension, and infection with a rat-borne hantavirus. Arch Virol 69-80.

Glass GE, Watson AJ, LeDuc JW, Kelen GD, Quinn TC and Childs JE (1993): Infection with a ratborne hantavirus in US residents is consistently associated with hypertensive renal disease. J Infect Dis 167:614-620.

Glass GE, Watson AJ, LeDuc JW and Childs JE (1994): Domestic cases of hemorrhagic fever with renal syndrome in the United States. Nephron 68:48-51.

Glassock RJ (1991): Immune complex-induced glomerular injury in viral diseases: an overview. Kidney Int Suppl 35:S5-7.

Glassock RJ, Cohen AH, Danovitch G and Parsa KP (1990): Human immunodeficiency virus (HIV) infection and the kidney. Ann Intern Med 112:35-49.

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Golbus SM and Wilson CB (1979): Experimental glomerulonephritis induced by in situ formation of immune complexes in glomerular capillary wall. Kidney Int 16:148-157.

Golovljova I, Sjölander KB, Lindegren G, Vene S, Vasilenko V, Plyusnin A and Lundkvist Å (2002): Hantaviruses in Estonia. J Med Virol 68:589-598.

Golovljova I, Vasilenko V, Mittzenkov V, Prükk T, Seppet E, Vene S, Settergren B, Plyusnin A and Lundkvist Å (2007): Characterization of hemorrhagic fever with renal syndrome caused by hantaviruses, Estonia. Emerg Infect Dis 13:1773-1776.

Grcevska L, Polenakovic M, Oncevski A, Zografski D and Gligic A (1990): Different pathohistological presentations of acute renal involvement in Hantaan virus infection: report of two cases. Clin Nephrol 34:197-201.

Groen J, Gerding M, Jordans JG, Clement JP and Osterhaus AD (1994): Class and subclass distribution of Hantavirus-specific serum antibodies at different times after the onset of nephropathia epidemica. J Med Virol 43:39-43.

Groen J, Bruijn JA, Gerding MN, Jordans JG, Moll van Charante AW and Osterhaus AD (1996): Hantavirus antigen detection in kidney biopsies from patients with nephropathia epidemica. Clin Nephrol 46:379-383.

Groeneveld PH, Colson P, Kwappenberg KM and Clement J (1995): Increased production of nitric oxide in patients infected with the European variant of hantavirus. Scand J Infect Dis 27:453-456.

Hallin GW, Simpson SQ, Crowell RE, James DS, Koster FT, Mertz GJ and Levy H (1996): Cardiopulmonary manifestations of hantavirus pulmonary syndrome. Crit Care Med 24:252-258.

Hardestam J, Simon M, Hedlund KO, Vaheri A, Klingström J and Lundkvist Å (2007): Ex vivo stability of the rodent-borne Hantaan virus in comparison to that of arthropod-borne members of the Bunyaviridae family. Appl Environ Microbiol 73:2547-2551.

Hardestam J, Karlsson M, Falk KI, Olsson G, Klingström J and Lundkvist Å (2008): Puumala hantavirus excretion kinetics in bank voles (Myodes glareolus). Emerg Infect Dis 14:1209-1215.

Hautala N, Kauma H, Vapalahti O, Mähönen SM, Vainio O, Vaheri A and Hautala T (2010): Prospective study on ocular findings in acute Puumala hantavirus infection in hospitalised patients. Br J Ophthalmol Aug 1 [epub ahead of print].

Hautala T, Sironen T, Vapalahti O, Pääkkö E, Särkioja T, Salmela PI, Vaheri A, Plyusnin A and Kauma H (2002): Hypophyseal hemorrhage and panhypopituitarism during Puumala Virus Infection: Magnetic Resonance Imaging and detection of viral antigen in the hypophysis. Clin Infect Dis 35:96-101.

Hautala T, Mähönen SM, Sironen T, Hautala N, Pääkkö E, Karttunen A, Salmela PI, Ilonen J, Vainio O, Glumoff V, Rytky S, Plyusnin A, Vaheri A, Vapalahti O and Kauma H (2010): Central nervous system-related symptoms and findings are common in acute Puumala hantavirus infection. Ann Med 42:344-351.

Hayasaka D, Maeda K, Ennis FA and Terajima M (2007): Increased permeability of human endothelial cell line EA.hy926 induced by hantavirus-specific cytotoxic T lymphocytes. Virus Res 123:120-127.

Hegedüs L (2001): Thyroid ultrasound. Endocrinol Metab Clin North Am 30:339-360.

Herget-Rosenthal S, Poppen D, Hüsing J, Marggraf G, Pietruck F, Jakob HG, Philipp T and Kribben A (2004): Prognostic value of tubular proteinuria and enzymuria in nonoliguric acute tubular necrosis. Clin Chem 50:552-558.

Heyman P and Vaheri A (2008): Situation of hantavirus infections and haemorrhagic fever with renal syndrome in European countries as of December 2006. Euro Surveill 13:pii 18925.

Heyman P, Vaheri A, Lundkvist Å and Avsic-Zupanc T (2009): Hantavirus infections in Europe: from virus carriers to a major public-health problem. Expert Rev Anti Infect Ther 7:205-217.

Hollowell JG, Staehling NW, Flanders WD, Hannon WH, Gunter EW, Spencer CA and Braverman LE (2002): Serum TSH, T(4), and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). J Clin Endocrinol Metab 87:489-499.

Huang C, Jin B, Wang M, Li E and Sun C (1994): Hemorrhagic fever with renal syndrome: relationship between pathogenesis and cellular immunity. J Infect Dis 169:868-870.

Huggins JW, Hsiang CM, Cosgriff TM, Guang MY, Smith JI, Wu ZO, LeDuc JW, Zheng ZM, Meegan JM, Wang QN and et al. (1991): Prospective, double-blind, concurrent, placebo-controlled clinical trial of intravenous ribavirin therapy of hemorrhagic fever with renal syndrome. J Infect Dis 164:1119-1127.

Hujakka H, Koistinen V, Eerikäinen P, Kuronen I, Mononen I, Parviainen M, Lundkvist Å, Vaheri A, Närvänen A and Vapalahti O (2001): New immunochromatographic rapid test for diagnosis of acute Puumala virus infection. J Clin Microbiol 39:2146-2150.

Hujakka H, Koistinen V, Kuronen I, Eerikäinen P, Parviainen M, Lundkvist Å, Vaheri A, Vapalahti O and Närvänen A (2003): Diagnostic rapid tests for acute hantavirus infections: specific tests for Hantaan, Dobrava and Puumala viruses versus a hantavirus combination test. J Virol Methods 108:117-122.

Hukic M, Valjevac A, Tulumovic D, Numanovic F and Heyman P (2010): Pathogenicity and virulence of the present hantaviruses in Bosnia and Herzegovina: the impact on renal function. Eur J Clin Microbiol Infect Dis Oct 23 [epub ahead of print].

Hulkkonen J, Pertovaara M, Antonen J, Pasternack A and Hurme M (2001): Elevated interleukin-6 plasma levels are regulated by the promoter region polymorphism of the IL6 gene in primary Sjogren's syndrome and correlate with the clinical manifestations of the disease. Rheumatology (Oxford) 40:656-661.

Hutchinson KL, Rollin PE and Peters CJ (1998): Pathogenesis of a North American hantavirus, Black Creek Canal virus, in experimentally infected Sigmodon hispidus. Am J Trop Med Hyg 59:58-65.

Huttunen NP, Mäkelä S, Pokka T, Mustonen J and Uhari M (2011): Systematic literature review of symptoms, signs and severity of serologically confirmed nephropathia epidemica in paediatric and adult patients. Scand J Infect Dis Early Online 1-6.

Hägg E, Åstrom L and Steen L (1978): Persistent hypothalamic-pituitary insufficiency following acute meningoencephalitis. A report of two cases. Acta Med Scand 203:231-235.

Härkönen K, Huhtaniemi I, Mäkinen J, Hübler D, Irjala K, Koskenvuo M, Oettel M, Raitakari O, Saad F and Pöllänen P (2003): The polymorphic androgen receptor gene CAG repeat, pituitary-testicular function and andropausal symptoms in ageing men. Int J Androl 26:187-194.

Hörling J, Lundkvist Å, Persson K, Mullaart M, Dzagurova T, Dekonenko A, Tkachenko E and Niklasson B (1995): Detection and subsequent sequencing of Puumala virus from human specimens by PCR. J Clin Microbiol 33:277-282.

Hörnfeldt B (2004): Long-term decline in numbers of cyclic voles in boreal Sweden: analysis and presentation of hypotheses. OIKOS 107:376-392.

Iglesias P and Diez JJ (2009): Thyroid dysfunction and kidney disease. Eur J Endocrinol 160:503-515.

Jiang H, Wang PZ, Zhang Y, Xu Z, Sun L, Wang LM, Huang CX, Lian JQ, Jia ZS, Li ZD and Bai XF (2008): Hantaan virus induces toll-like receptor 4 expression, leading to enhanced production of beta interferon, interleukin-6 and tumor necrosis factor-alpha. Virology 380:52-59.

Jin HY, Kang SM, Kim SY, Park JH, Baek HS and Park TS (2009): A case of Graves' disease combined with hantaan virus infection. J Korean Med Sci 24:158-161.

Johnson KM (2001): Hantaviruses: History and overview. Curr Top Microbiol Immunol 256:1-14.

Johnson RJ, Herrera-Acosta J, Schreiner GF and Rodriguez-Iturbe B (2002): Subtle acquired renal injury as a mechanism of salt-sensitive hypertension. N Engl J Med 346:913-923.

Johnson RJ, Feig DI, Nakagawa T, Sanchez-Lozada LG and Rodriguez-Iturbe B (2008): Pathogenesis of essential hypertension: historical paradigms and modern insights. J Hypertens 26:381-391.

Jokinen EJ, Lähdevirta J and Collan Y (1978): Nephropathia epidemica: immunohistochemical study of pathogenesis. Clin Nephrol 9:1-5.

Jonsson CB, Figueiredo LT and Vapalahti O (2010): A global perspective on hantavirus ecology, epidemiology, and disease. Clin Microbiol Rev 23:412-441.

Jurney TH, Cockrell JL, Jr., Lindberg JS, Lamiell JM and Wade CE (1987): Spectrum of serum cortisol response to ACTH in ICU patients. Correlation with degree of illness and mortality. Chest 92:292-295.

Kallio ER, Klingström J, Gustafsson E, Manni T, Vaheri A, Henttonen H, Vapalahti O and Lundkvist Å (2006): Prolonged survival of Puumala hantavirus outside the host: evidence for indirect transmission via the environment. J Gen Virol 87:2127-2134.

Kanerva M, Paakkala A, Mustonen J, Paakkala T, Lahtela J and Pasternack A (1996): Pulmonary involvement in nephropathia epidemica: radiological findings and their clinical correlations. Clin Nephrol 46:369-378.

Kanerva M, Mustonen J and Vaheri A (1998a): Pathogenesis of puumala and other hantavirus infections. Rev Med Virol 8:67-86.

Kanerva M, Vaheri A, Mustonen J and Partanen J (1998b): High-producer allele of tumour necrosis factor-alpha is part of the susceptibility MHC haplotype in severe puumala virus-induced nephropathia epidemica. Scand J Infect Dis 30:532-534.

Kaptein EM, Levitan D, Feinstein EI, Nicoloff JT and Massry SG (1981): Alterations of thyroid hormone indices in acute renal failure and in acute critical illness with and without acute renal failure. Am J Nephrol 1:138-143.

Kaptein EM (1996): Thyroid hormone metabolism and thyroid diseases in chronic renal failure. Endocr Rev 17:45-63.

Karamadoukis L, Toth T and Tomson C (2008): Membranoproliferative glomerulonephritis associated with an Epstein-Barr virus infection. NDT Plus 5:319-321.

Kilpatrick ED, Terajima M, Koster FT, Catalina MD, Cruz J and Ennis FA (2004): Role of specific CD8+ T cells in the severity of a fulminant zoonotic viral hemorrhagic fever, hantavirus pulmonary syndrome. J Immunol 172:3297-3304.

Kim NH, Cho JG, Ahn YK, Lee SU, Kim KH, Cho JH, Kim HG, Kim W, Jeong MH, Park JC and Kang JC (2001): A case of torsade de pointes associated with hypopituitarism due to hemorrhagic fever with renal syndrome. J Korean Med Sci 16:355-359.

Kim S, Kang ET, Kim YG, Han JS, Lee JS, Kim YI, Hall WC, Dalrymple JM and Peters CJ (1993): Localization of Hantaan viral envelope glycoproteins by monoclonal antibodies in renal tissues from patients with Korean hemorrhagic fever H. Am J Clin Pathol 100:398-403.

Kim S, Sung SH, An HR, Jun YH, Yu M, Ryu DR, Kim SJ, Kang DH and Choi KB (2010): A case report of crescentic glomerulonephritis associated with Hantaan virus infection. Nephrol Dial Transplant 25:2790-2792.

Kim SH, Kim S, Lee JS, Choi BI, Kim YG, Han JS, Park JH, Han MC and Kim CW (1990): Hemorrhagic fever with renal syndrome: MR imaging of the kidney. Radiology 175:823-825.

Kleinknecht D and Rollin PE (1992): Hypertension after hemorrhagic fever with renal syndrome. Nephron 61:121.

Klingström J, Plyusnin A, Vaheri A and Lundkvist Å (2002): Wild-type Puumala hantavirus infection induces cytokines, C-reactive protein, creatinine, and nitric oxide in cynomolgus macaques. J Virol 76:444-449.

Klingström J, Hardestam J, Stoltz M, Zuber B, Lundkvist Å, Linder S and Ahlm C (2006): Loss of cell membrane integrity in puumala hantavirus-infected patients correlates with levels of epithelial cell apoptosis and perforin. J Virol 80:8279-8282.

Klingström J, Lindgren T and Ahlm C (2008): Sex-dependent differences in plasma cytokine responses to hantavirus infection. Clin Vaccine Immunol 15:885-887.

Kokot F, Mleczko Z and Pazera A (1982): Parathyroid hormone, prolactin, and function of the pituitary-gonadal axis in male patients with acute renal failure. Kidney Int 21:84-89.

Kontkanen M, Puustjärvi T and Lähdevirta J (1994): Myopic shift and its mechanism in nephropathia epidemica or Puumala virus infection. Br J Ophthalmol 78:903-906.

Krakauer T, Leduc JW, Morrill JC, Anderson AO and Krakauer H (1994): Serum levels of alpha and gamma interferons in hemorrhagic fever with renal syndrome. Viral Immunol 7:97-101.

Krakauer T, Leduc JW and Krakauer H (1995): Serum levels of tumor necrosis factor-alpha, interleukin-1, and interleukin-6 in hemorrhagic fever with renal syndrome. Viral Immunol 8:75-79.

Krause R, Aberle S, Haberl R, Daxböck F and Wenisch C (2003): Puumala virus infection with acute disseminated encephalomyelitis and multiorgan failure. Emerg Infect Dis 9:603-605.

Krautkrämer E and Zeier M (2008): Hantavirus causing hemorrhagic fever with renal syndrome enters from the apical surface and requires decay-accelerating factor (DAF/CD55). J Virol 82:4257-4264.

Kuhlbäck B, Fortelius P and Tallgren LG (1964): Renal Histopathology in a Case of Nephropathia Epidemica Myhrman. A Study of Successive Biopsies. Acta Pathol Microbiol Scand 60:323-333.

Kupari M, Pelkonen R and Valtonen V (1980): Post-encephalitic hypothalamic-pituitary insufficiency. Acta Endocrinol (Copenh) 94:433-438.

Laine O, Mäkelä S, Mustonen J, Huhtala H, Szanto T, Vaheri A, Lassila R and Joutsi-Korhonen L (2010): Enhanced thrombin formation and fibrinolysis during acute Puumala hantavirus infection. Thromb Res 126:154-158.

Laine O, Makela S, Mustonen J, Helminen M, Vaheri A, Lassila R and Joutsi-Korhonen L (2011): Platelet ligands and ADAMTS13 during Puumala hantavirus infection and associated thrombocytopenia. Blood Coagul Fibrinolysis DOI: 10.1097/MBC.0b013e328346a420.

Larson RS, Brown DC, Ye C and Hjelle B (2005): Peptide antagonists that inhibit Sin Nombre virus and hantaan virus entry through the beta3-integrin receptor. J Virol 79:7319-7326.

Launay D, Thomas C, Fleury D, Roueff S, Line ML, Droz D and Vanhille P (2003): Pulmonary-renal syndrome due to hemorrhagic fever with renal syndrome: an unusual manifestation of puumala virus infection in France. Clin Nephrol 59:297-300.

Launes J and Hautanen A (1988): Nephropathia epidemica encephalitis. Acta Neurol Scand 78:234-235.

Ledina D, Bradaric N, Ivic I, Marasovic D, Radovic D, Ostojic-Bakotin V and Prgomet S (2003): [Is permanent renal function damage possible after hemorrhagic fever with renal syndrome?]. Acta Med Croatica 57:365-368.

Lee HW (1982a): Hemorrhagic fever with renal syndrome (HFRS). Scand J Infect Dis Suppl 36:82-85.

Lee HW and van der Groen G (1989): Hemorrhagic fever with renal syndrome. Prog Med Virol 36:62-102.

Lee HW, Lee PW and Johnson KM (1978): Isolation of the etiologic agent of Korean Hemorrhagic fever. J Infect Dis 137:298-308.

Lee HW, Lee PW, Baek LJ, Song CK and Seong IW (1981): Intraspecific transmission of Hantaan virus, etiologic agent of Korean hemorrhagic fever, in the rodent Apodemus agrarius. Am J Trop Med Hyg 30:1106-1112.

Lee HW, Baek LJ and Johnson KM (1982b): Isolation of Hantaan virus, the etiologic agent of Korean hemorrhagic fever, from wild urban rats. J Infect Dis 146:638-644.

Levin A (1999): Management of membranoproliferative glomerulonephritis: evidence-based recommendations. Kidney Int Suppl 70:S41-46.

Levitan D, Moser SA, Goldstein DA, Kletzky OA, Lobo RA and Massry SG (1984): Disturbances in the hypothalamic-pituitary-gonadal axis in male patients with acute renal failure. Am J Nephrol 4:99-106.

Lim TH, Chang KH, Han MC, Chang YB, Lim SM, Yu YS, Chun YH and Lee JS (1986): Pituitary atrophy in Korean (epidemic) hemorrhagic fever: CT correlation with pituitary function and visual field. AJNR Am J Neuroradiol 7:633-637.

Linderholm M, Settergren B, Ahlm C, Burman LA, Träff S, Bäcklund U and Juto P (1991): A Swedish fatal case of nephropathia epidemica. Scand J Infect Dis 23:501-502.

Linderholm M, Bjermer L, Juto P, Roos G, Sandström T, Settergren B and Tärnvik A (1993): Local host response in the lower respiratory tract in nephropathia epidemica. Scand J Infect Dis 25:639-646.

Linderholm M, Ahlm C, Settergren B, Waage A and Tärnvik A (1996a): Elevated plasma levels of tumor necrosis factor (TNF)-alpha, soluble TNF receptors, interleukin (IL)-6, and IL-10 in patients with hemorrhagic fever with renal syndrome. J Infect Dis 173:38-43.

Linderholm M, Groeneveld PH and Tarnvik A (1996b): Increased production of nitric oxide in patients with hemorrhagic fever with renal syndrome - relation to arterial hypotension and tumor necrosis factor. Infection 24:337-340.

Low MJ (2007): Hypophyseotropic hormones and neuroendocrine axes. In: Williams Textbook of Endocrinology, pp. 96-136. Eds. Kronenberg HM, Saunders Elsevier, Philadelphia.

Lundkvist Å, Hörling J and Niklasson B (1993a): The humoral response to Puumala virus infection (nephropathia epidemica) investigated by viral protein specific immunoassays. Arch Virol 130:121-130.

Lundkvist Å, Björsten S and Niklasson B (1993b): Immunoglobulin G subclass responses against the structural components of Puumala virus. J Clin Microbiol 31:368-372.

Lundkvist Å, Kallio-Kokko H, Sjölander KB, Lankinen H, Niklasson B, Vaheri A and Vapalahti O (1996): Characterization of Puumala virus nucleocapsid protein: identification of B-cell epitopes and domains involved in protective immunity. Virology 216:397-406.

Lundkvist Å, Hukic M, Hörling J, Gilljam M, Nichol S and Niklasson B (1997): Puumala and Dobrava viruses cause hemorrhagic fever with renal syndrome in Bosnia-Herzegovina: evidence of highly cross-neutralizing antibody responses in early patient sera. J Med Virol 53:51-59.

Lähdevirta J (1971): Nephropathia epidemica in Finland. A clinical histological and epidemiological study. Ann Clin Res 3:1-54.

Lähdevirta J and Savola J (1985): Nephropathia epidemicasta myyräkuumeeseen. Duodecim 6:78-80.

Lähdevirta J, Collan Y, Jokinen EJ and Hiltunen R (1978): Renal sequelae to nephropathia epidemica. Acta Pathol Microbiol Scand [A] 86:265-271.

Mackow ER and Gavrilovskaya IN (2001): Cellular receptors and hantavirus pathogenesis. Curr Top Microbiol Immunol 256:91-115.

Mackow ER and Gavrilovskaya IN (2009): Hantavirus regulation of endothelial cell functions. Thromb Haemost 102:1030-1041.

Maes P, Clement J, Gavrilovskaya I and Van Ranst M (2004): Hantaviruses: immunology, treatment, and prevention. Viral Immunol 17:481-497.

Maes P, Clement J, Groeneveld PH, Colson P, Huizinga TW and Van Ranst M (2006): Tumor necrosis factor-alpha genetic predisposing factors can influence clinical severity in nephropathia epidemica. Viral Immunol 19:558-564.

Maes P, Clement J, Cauwe B, Bonnet V, Keyaerts E, Robert A and Van Ranst M (2008): Truncated recombinant puumala virus nucleocapsid proteins protect mice against challenge in vivo. Viral Immunol 21:49-60.

Makary P, Kanerva M, Ollgren J, Virtanen MJ, Vapalahti O and Lyytikäinen O (2010): Disease burden of Puumala virus infections, 1995-2008. Epidemiol Infect 138:1484-1492.

Manigold T, Mori A, Graumann R, Llop E, Simon V, Ferres M, Valdivieso F, Castillo C, Hjelle B and Vial P (2010): Highly differentiated, resting gn-specific memory CD8+ T cells persist years after infection by andes hantavirus. PLoS Pathog 6:e1000779.

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Markotic A, Dasic G, Gagro A, Sabioncello A, Rabatic S, Kuzman I, Zgorelec R, Smoljan I, Beus I, Zupanc TA and Dekaris D (1999): Role of peripheral blood mononuclear cell (PBMC) phenotype changes in the pathogenesis of haemorrhagic fever with renal syndrome (HFRS). Clin Exp Immunol 115:329-334.

Markotic A, Nichol ST, Kuzman I, Sanchez AJ, Ksiazek TG, Gagro A, Rabatic S, Zgorelec R, Avsic-Zupanc T, Beus I and Dekaris D (2002): Characteristics of Puumala and Dobrava infections in Croatia. J Med Virol 66:542-551.

Markotic A, Hensley L, Daddario K, Spik K, Anderson K and Schmaljohn C (2007): Pathogenic hantaviruses elicit different immunoreactions in THP-1 cells and primary monocytes and induce differentiation of human monocytes to dendritic-like cells. Coll Antropol 31:1159-1167.

Mertz GJ, Miedzinski L, Goade D, Pavia AT, Hjelle B, Hansbarger CO, Levy H, Koster FT, Baum K, Lindemulder A, Wang W, Riser L, Fernandez H and Whitley RJ (2004): Placebo-controlled, double-blind trial of intravenous ribavirin for the treatment of hantavirus cardiopulmonary syndrome in North America. Clin Infect Dis 39:1307-1313.

Meyers CM, Seeff LB, Stehman-Breen CO and Hoofnagle JH (2003): Hepatitis C and renal disease: an update. Am J Kidney Dis 42:631-657.

Mori K and Yoshida K (2010): Viral infection in induction of Hashimoto's thyroiditis: a key player or just a bystander? Curr Opin Endocrinol Diabetes Obes 17:418-424.

Mori M, Rothman AL, Kurane I, Montoya JM, Nolte KB, Norman JE, Waite DC, Koster FT and Ennis FA (1999): High levels of cytokine-producing cells in the lung tissues of patients with fatal hantavirus pulmonary syndrome. J Infect Dis 179:295-302.

Muranyi W, Bahr U, Zeier M and van der Woude FJ (2005): Hantavirus infection. J Am Soc Nephrol 16:3669-3679.

Mustonen J, Brummer-Korvenkontio M, Hedman K, Pasternack A, Pietilä K and Vaheri A (1994a): Nephropathia epidemica in Finland: a retrospective study of 126 cases. Scand J Infect Dis 26:7-13.

Mustonen J, Helin H, Pietilä K, Brummer-Korvenkontio M, Hedman K, Vaheri A and Pasternack A (1994b): Renal biopsy findings and clinicopathologic correlations in nephropathia epidemica. Clin Nephrol 41:121-126.

Mustonen J, Huttunen NP, Brummer-Korvenkontio M and Vaheri A (1994c): Clinical picture of nephropathia epidemica in children. Acta Paediatr 83:526-529.

Mustonen J, Partanen J, Kanerva M, Pietilä K, Vapalahti O, Pasternack A and Vaheri A (1996): Genetic susceptibility to severe course of nephropathia epidemica caused by Puumala hantavirus. Kidney Int 49:217-221.

Mustonen J, Partanen J, Kanerva M, Pietilä K, Vapalahti O, Pasternack A and Vaheri A (1998): Association of HLA B27 with benign clinical course of nephropathia epidemica caused by Puumala hantavirus. Scand J Immunol 47:277-279.

Mustonen J, Mäkelä S, Helin H, Helanterä A, Miettinen M, Partanen J and Pasternack A (2001): Mesangiocapillary glomerulonephritis caused by Puumala hantavirus infection. Nephron 89:402-407.

Myhrman G (1934): En njursjukdom med egenartad symptombild. Nord Med Tidskr 7:793-794.

Mähönen SM, Sironen T, Vapalahti O, Pääkkö E, Hautala N, Ilonen J, Glumoff V, Vainio O, Kauma H, Vaheri A, Plyusnin A and Hautala T (2007): Puumala virus RNA in cerebrospinal fluid in a patient with uncomplicated nephropathia epidemica. J Clin Virol 40:248-251.

Mäkelä S, Ala-Houhala I, Mustonen J, Koivisto AM, Kouri T, Turjanmaa V, Vapalahti O, Vaheri A and Pasternack A (2000): Renal function and blood pressure five years after puumala virus-induced nephropathy. Kidney Int 58:1711-1718.

Mäkelä S, Hurme M, Ala-Houhala I, Mustonen J, Koivisto AM, Partanen J, Vapalahti O, Vaheri A and Pasternack A (2001): Polymorphism of the cytokine genes in hospitalized patients with Puumala hantavirus infection. Nephrol Dial Transplant 16:1368-1373.

Mäkelä S, Mustonen J, Ala-Houhala I, Hurme M, Partanen J, Vapalahti O, Vaheri A and Pasternack A (2002): Human leukocyte antigen-B8-DR3 is a more important risk factor

for severe Puumala hantavirus infection than the tumor necrosis factor-alpha(-308) G/A polymorphism. J Infect Dis 186:843-846.

Mäkelä S, Mustonen J, Ala-Houhala I, Hurme M, Koivisto AM, Vaheri A and Pasternack A (2004): Urinary excretion of interleukin-6 correlates with proteinuria in acute Puumala hantavirus-induced nephritis. Am J Kidney Dis 43:809-816.

Mäkelä S, Kokkonen L, Ala-Houhala I, Groundström K, Harmoinen A, Huhtala H, Hurme M, Paakkala A, Pörsti I, Virtanen V, Vaheri A and Mustonen J (2009): More than half of the patients with acute Puumala hantavirus infection have abnormal cardiac findings. Scand J Infect Dis 41:57-62.

Nakamura I, Yoshimatsu K, Lee BH, Okumura M, Taruishi M, Araki K, Kariwa H, Takashima I and Arikawa J (2008): Development of a serotyping ELISA system for Thailand virus infection. Arch Virol 153:1537-1542.

Nakopoulou L (2001): Membranoproliferative glomerulonephritis. Nephrol Dial Transplant 16 Suppl 6:71-73.

National Institute for Health and Welfare, Finland. http://www.zoonoosikeskus.fi/attachments/zoonoosit/myyr_kuume/myyrakuume_1_110321pdf.pdf (accessed May 2011).

Nemirov K, Vapalahti O, Lundkvist Å, Vasilenko V, Golovljova I, Plyusnina A, Niemimaa J, Laakkonen J, Henttonen H, Vaheri A and Plyusnin A (1999): Isolation and characterization of Dobrava hantavirus carried by the striped field mouse (Apodemus agrarius) in Estonia. J Gen Virol 80 (Pt 2):371-379.

Nemirov K, Henttonen H, Vaheri A and Plyusnin A (2002): Phylogenetic evidence for host switching in the evolution of hantaviruses carried by Apodemus mice. Virus Res 90:207-215.

Nichol ST, Spiropoulou CF, Morzunov S, Rollin PE, Ksiazek TG, Feldmann H, Sanchez A, Childs J, Zaki S and Peters CJ (1993): Genetic identification of a hantavirus associated with an outbreak of acute respiratory illness. Science 262:914-917.

Niikura M, Maeda A, Ikegami T, Saijo M, Kurane I and Morikawa S (2004): Modification of endothelial cell functions by Hantaan virus infection: prolonged hyper-permeability induced by TNF-alpha of hantaan virus-infected endothelial cell monolayers. Arch Virol 149:1279-1292.

Niklasson B and LeDuc JW (1987): Epidemiology of nephropathia epidemica in Sweden. J Infect Dis 155:269-276.

Niklasson B, Hellsten G and LeDuc J (1994): Hemorrhagic fever with renal syndrome: a study of sequelae following nephropathia epidemica. Arch Virol 137:241-247.

Nolte KB, Feddersen RM, Foucar K, Zaki SR, Koster FT, Madar D, Merlin TL, McFeeley PJ, Umland ET and Zumwalt RE (1995): Hantavirus pulmonary syndrome in the United States: a pathological description of a disease caused by a new agent. Hum Pathol 26:110-120.

Novo R, Gagnadoux MF, Le Guenno Y, Gubler MC, Niaudet P, Guyot C and Broyer M (1999): Chronic renal failure after Puumala virus infection. Pediatr Nephrol 13:934-935.

Nuutinen H, Vuoristo M, Färkkilä M, Kahri A, Seppälä K, Valtonen V, Joutsiniemi T and Miettinen T (1992): Hemorrhagic gastropathy in epidemic nephropathy. Gastrointest Endosc 38:476-480.

Olsson GE, Dalerum F, Hörnfeldt B, Elgh F, Palo TR, Juto P and Ahlm C (2003): Human hantavirus infections, Sweden. Emerg Infect Dis 9:1395-1401.

Orth SR and Ritz E (1998): The nephrotic syndrome. N Engl J Med 338:1202-1211.

Outinen TK, Mäkelä SM, Ala-Houhala IO, Huhtala HS, Hurme M, Paakkala AS, Pörsti IH, Syrjänen JT and Mustonen JT (2010): The severity of Puumala hantavirus induced nephropathia epidemica can be better evaluated using plasma interleukin-6 than C-reactive protein determinations. BMC Infect Dis 10:132.

Outinen TK, Makela SM, Ala-Houhala IO, Huhtala HS, Hurme M, Libraty DH, Oja SS, Porsti IH, Syrjanen JT, Vaheri A and Mustonen JT (2011): High activity of indoleamine 2,3-dioxygenase is associated with renal insufficiency in Puumala hantavirus induced nephropathia epidemica. J Med Virol 83:731-737.

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Paakkala A, Mustonen J, Viander M, Huhtala H and Pasternack A (2000): Complement activation in nephropathia epidemica caused by Puumala hantavirus. Clin Nephrol 53:424-431.

Paakkala A, Kallio T, Huhtala H, Apuli P, Paakkala T, Pasternack A and Mustonen J (2002): Renal ultrasound findings and their clinical associations in nephropathia epidemica. Analysis of quantitative parameters. Acta Radiol 43:320-325.

Paakkala A, Lempinen L, Paakkala T, Huhtala H and Mustonen J (2004): Medical imaging in nephropathia epidemica and their clinical correlations. Eur J Intern Med 15:284-290.

Paakkala A, Dastidar P, Ryymin P, Huhtala H and Mustonen J (2005): Renal MRI findings and their clinical associations in nephropathia epidemica: analysis of quantitative findings. Eur Radiol 15:968-974.

Paakkala A, Ryymin P, Dastidar P, Huhtala H and Mustonen J (2006): Magnetic resonance renography findings and their clinical associations in nephropathia epidemica. Acta Radiol 47:213-221.

Paakkala A, Mäkelä S, Hurme M, Partanen J, Huhtala H and Mustonen J (2008): Association of chest radiography findings with host-related genetic factors in patients with nephropathia epidemica. Scand J Infect Dis 40:254-258.

Padula PJ, Edelstein A, Miguel SD, Lopez NM, Rossi CM and Rabinovich RD (1998): Hantavirus pulmonary syndrome outbreak in Argentina: molecular evidence for person-to-person transmission of Andes virus. Virology 241:323-330.

Palatini P, Mormino P, Dorigatti F, Santonastaso M, Mos L, De Toni R, Winnicki M, Dal Follo M, Biasion T, Garavelli G and Pessina AC (2006): Glomerular hyperfiltration predicts the development of microalbuminuria in stage 1 hypertension: the HARVEST. Kidney Int 70:578-584.

Parikh CR, Lu JC, Coca SG and Devarajan P (2010): Tubular proteinuria in acute kidney injury: a critical evaluation of current status and future promise. Ann Clin Biochem 47:301-312.

Park JE and Pyo HJ (1996): Delayed onset of diuresis in a patient with acute renal failure due to hemorrhagic fever with renal syndrome who also developed anterior hypopituitarism. Clin Nephrol 46:141-145.

Pattamadilok S, Lee BH, Kumperasart S, Yoshimatsu K, Okumura M, Nakamura I, Araki K, Khoprasert Y, Dangsupa P, Panlar P, Jandrig B, Krüger DH, Klempa B, Jäkel T, Schmidt J, Ulrich R, Kariwa H and Arikawa J (2006): Geographical distribution of hantaviruses in Thailand and potential human health significance of Thailand virus. Am J Trop Med Hyg 75:994-1002.

Peeters RP, Wouters PJ, Kaptein E, van Toor H, Visser TJ and Van den Berghe G (2003): Reduced activation and increased inactivation of thyroid hormone in tissues of critically ill patients. J Clin Endocrinol Metab 88:3202-3211.

Pekic S, Cvijovic G, Stojanovic M, Kendereski A, Micic D and Popovic V (2005): Hypopituitarism as a late complication of hemorrhagic fever. Endocrine 26:79-82.

Pensiero MN, Sharefkin JB, Dieffenbach CW and Hay J (1992): Hantaan virus infection of human endothelial cells. J Virol 66:5929-5936.

Penttinen K, Lähdevirta J, Kekomäki R, Ziola B, Salmi A, Hautanen A, Lindström P, Vaheri A, Brummer-Korvenkontio M and Wager O (1981): Circulating immune complexes, immunoconglutinins, and rheumatoid factors in nephropathia epidemica. J Infect Dis 143:15-21.

Pergam SA, Schmidt DW, Nofchissey RA, Hunt WC, Harford AH and Goade DE (2009): Potential renal sequelae in survivors of hantavirus cardiopulmonary syndrome. Am J Trop Med Hyg 80:279-285.

Peters CJ, Simpson GL and Levy H (1999): Spectrum of hantavirus infection: hemorrhagic fever with renal syndrome and hantavirus pulmonary syndrome. Annu Rev Med 50:531-545.

Pettersson L, Boman J, Juto P, Evander M and Ahlm C (2008a): Outbreak of Puumala virus infection, Sweden. Emerg Infect Dis 14:808-810.

Pettersson L, Klingström J, Hardestam J, Lundkvist Å, Ahlm C and Evander M (2008b): Hantavirus RNA in saliva from patients with hemorrhagic fever with renal syndrome. Emerg Infect Dis 14:406-411.

Pierzchalski P, Rokita H, Koj A, Fries E and Åkerstrom B (1992): Synthesis of alpha 1-microglobulin in cultured rat hepatocytes is stimulated by interleukin-6, leukemia inhibitory factor, dexamethasone and retinoic acid. FEBS Lett 298:165-168.

Plyusnin A (2002): Genetics of hantaviruses: implications to taxonomy. Arch Virol 147:665-682.

Plyusnin A and Morzunov SP (2001): Virus evolution and genetic diversity of hantaviruses and their rodent hosts. Curr Top Microbiol Immunol 256:47-75.

Plyusnin A, Vapalahti O, Lankinen H, Lehväslaiho H, Apekina N, Myasnikov Y, Kallio-Kokko H, Henttonen H, Lundkvist Å, Brummer-Korvenkontio M, Gavrilovskaya I and Vaheri A (1994): Tula virus: a newly detected hantavirus carried by European common voles. J Virol 68:7833-7839.

Plyusnin A, Vapalahti O and Vaheri A (1996): Hantaviruses: genome structure, expression and evolution. J Gen Virol 77 (Pt 11):2677-2687.

Plyusnin A, Vapalahti O, Vasilenko V, Henttonen H and Vaheri A (1997a): Dobrava hantavirus in Estonia: does the virus exist throughout Europe? Lancet 349:1369-1370.

Plyusnin A, Hörling J, Kanerva M, Mustonen J, Cheng Y, Partanen J, Vapalahti O, Kukkonen SK, Niemimaa J, Henttonen H, Niklasson B, Lundkvist Å and Vaheri A (1997b): Puumala hantavirus genome in patients with nephropathia epidemica: correlation of PCR positivity with HLA haplotype and link to viral sequences in local rodents. J Clin Microbiol 35:1090-1096.

Plyusnin A, Mustonen J, Asikainen K, Plyusnina A, Niemimaa J, Henttonen H and Vaheri A (1999): Analysis of puumala hantavirus genome in patients with nephropathia epidemica and rodent carriers from the sites of infection. J Med Virol 59:397-405.

Poljak M and Avsic Zupanc T (1994): Immunohistochemical detection of Hantaan virus antigen in renal tissue from patient with hemorrhagic fever with renal syndrome. Nephron 67:252.

Ponticelli C (2007): Membranous nephropathy. J Nephrol 20:268-287.

Praga M and Gonzalez E (2010): Acute interstitial nephritis. Kidney Int 77:956-961.

Puljiz I, Kuzman I, Markotic A, Turcinov D, Matic M and Makek N (2005): Electrocardiographic changes in patients with haemorrhagic fever with renal syndrome. Scand J Infect Dis 37:594-598.

Raftery MJ, Kraus AA, Ulrich R, Krüger DH and Schönrich G (2002): Hantavirus infection of dendritic cells. J Virol 76:10724-10733.

Rasmuson J, Andersson C, Norrman E, Haney M, Evander M and Ahlm C (2011a): Time to revise the paradigm of hantavirus syndromes? Hantavirus pulmonary syndrome caused by European hantavirus. Eur J Clin Microbiol Infect Dis Jan 15 Epub ahead of print.

Rasmuson J, Pourazar J, Linderholm M, Sandstrom T, Blomberg A and Ahlm C (2011b): Presence of Activated Airway T Lymphocytes in Human Puumala Hantavirus Disease. Chest Mar 24 Epub ahead of print.

Ribstein J, du Cailar G and Mimran A (1995): Combined renal effects of overweight and hypertension. Hypertension 26:610-615.

Riquelme R, Riquelme M, Torres A, Rioseco ML, Vergara JA, Scholz L and Carriel A (2003): Hantavirus pulmonary syndrome, southern Chile. Emerg Infect Dis 9:1438-1443.

Rubini ME, Jablon S and Mc DM (1960): Renal residuals of acute epidemic hemorrhagic fever. Arch Intern Med 106:378-387.

Rusnak JM, Byrne WR, Chung KN, Gibbs PH, Kim TT, Boudreau EF, Cosgriff T, Pittman P, Kim KY, Erlichman MS, Rezvani DF and Huggins JW (2009): Experience with intravenous ribavirin in the treatment of hemorrhagic fever with renal syndrome in Korea. Antiviral Res 81:68-76.

Saggioro FP, Rossi MA, Duarte MI, Martin CC, Alves VA, Moreli ML, Figueiredo LT, Moreira JE, Borges AA and Neder L (2007): Hantavirus infection induces a typical myocarditis that may be responsible for myocardial depression and shock in hantavirus pulmonary syndrome. J Infect Dis 195:1541-1549.

Saltevo J and Forslund T (1992): [Epidemic nephritis followed by hypogonadism]. Duodecim 108:494-496.

Sane T and Färkkilä M (2002): [Hypopituitarism and hepatitis as complications of nephropathia epidemica]. Duodecim 118:457-461.

Sane J, Laine O, Makela S, Paakkala A, Jarva H, Mustonen J, Vapalahti O, Meri S and Vaheri A (2011): Complement activation in Puumala hantavirus infection correlates with disease severity. Ann Med DOI: 10.3109/07853890.2011.573500.

Schaefer S, Boegershausen N, Meyer S, Ivan D, Schepelmann K and Kann PH (2008): Hypothalamic-pituitary insufficiency following infectious diseases of the central nervous system. Eur J Endocrinol 158:3-9.

Scharninghausen JJ, Faulde M and Cavaljuga S (2004): Hantavirus host/virus interactions within Southeast Europe. Bosn J Basic Med Sci 4:13-18.

Schmaljohn C (2009): Vaccines for hantaviruses. Vaccine 27 Suppl 4:D61-64.

Schmaljohn C and Hjelle B (1997): Hantaviruses: a global disease problem. Emerg Infect Dis 3:95-104.

Schmieder RE, Veelken R, Schobel H, Dominiak P, Mann JF and Luft FC (1997): Glomerular hyperfiltration during sympathetic nervous system activation in early essential hypertension. J Am Soc Nephrol 8:893-900.

Schmitt H, Bohle A, Reineke T, Mayer-Eichberger D and Vogl W (1990): Long-term prognosis of membranoproliferative glomerulonephritis type I. Significance of clinical and morphological parameters: an investigation of 220 cases. Nephron 55:242-250.

Schultze D, Lundkvist Å, Blauenstein U and Heyman P (2002): Tula virus infection associated with fever and exanthema after a wild rodent bite. Eur J Clin Microbiol Infect Dis 21:304-306.

Schönrich G, Rang A, Lutteke N, Raftery MJ, Charbonnel N and Ulrich RG (2008): Hantavirus-induced immunity in rodent reservoirs and humans. Immunol Rev 225:163-189.

Segerer S, Nelson PJ and Schlondorff D (2000): Chemokines, chemokine receptors, and renal disease: from basic science to pathophysiologic and therapeutic studies. J Am Soc Nephrol 11:152-176.

Seitsonen E, Hynninen M, Kolho E, Kallio-Kokko H and Pettilä V (2006): Corticosteroids combined with continuous veno-venous hemodiafiltration for treatment of hantavirus pulmonary syndrome caused by Puumala virus infection. Eur J Clin Microbiol Infect Dis 25:261-266.

Settergren B, Juto P, Trollfors B, Wadell G and Norrby SR (1988): Hemorrhagic complications and other clinical findings in nephropathia epidemica in Sweden: a study of 355 serologically verified cases. J Infect Dis 157:380-382.

Settergren B, Juto P, Trollfors B, Wadell G and Norrby SR (1989): Clinical characteristics of nephropathia epidemica in Sweden: prospective study of 74 cases. Rev Infect Dis 11:921-927.

Settergren B, Trollfors B, Fasth A, Hultberg B and Norrby SR (1990): Glomerular filtration rate and tubular involvement during acute disease and convalescence in patients with nephropathia epidemica. J Infect Dis 161:716-720.

Settergren B, Boman J, Linderholm M, Wiström J, Hägg E and Arvidsson PA (1992): A case of nephropathia epidemica associated with panhypopituitarism and nephrotic syndrome. Nephron 61:234-235.

Simpson SQ, Spikes L, Patel S and Faruqi I (2010): Hantavirus pulmonary syndrome. Infect Dis Clin North Am 24:159-173.

Sinisalo M, Vapalahti O, Ekblom-Kullberg S, Laine O, Makela S, Rintala H and Vaheri A (2010): Headache and low platelets in a patient with acute leukemia. J Clin Virol 48:159-161.

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Sironen T, Klingström J, Vaheri A, Andersson LC, Lundkvist Å and Plyusnin A (2008): Pathology of Puumala hantavirus infection in macaques. PLoS One 3:e3035.

Smadel JE (1953): Epidemic hemorrhagic fever. Am J Public Health Nations Health 43:1327-1330.

Spiropoulou CF (2001): Hantavirus maturation. Curr Top Microbiol Immunol 256:33-46.

Spratt DI, Cox P, Orav J, Moloney J and Bigos T (1993): Reproductive axis suppression in acute illness is related to disease severity. J Clin Endocrinol Metab 76:1548-1554.

Staessen JA, O'Brien ET, Amery AK, Atkins N, Baumgart P, De Cort P, Degaute JP, Dolenc P, De Gaudemaris R, Enström I, Fagard R, Gosse P, Gourlay S, Hayashi H, Imai Y, James G, Kawasaki T, Kuschnir E, Kuwajima I, Lindholm L, Liu L, Macor F, Mancia G, McGrath B, Middeke M, Ming J, Omboni S, Otsuka K, Palatini P, Parati G, Pieper C, Verdecchia P, Zachariah P and Zhang W (1994): Ambulatory blood pressure in normotensive and hypertensive subjects: results from an international database. J Hypertens Suppl 12:S1-12.

Stojanovic M, Pekic S, Cvijovic G, Miljic D, Doknic M, Nikolic-Djurovic M, Micic D, Hrvacevic R, Nesic V and Popovic V (2008): High risk of hypopituitarism in patients who recovered from hemorrhagic fever with renal syndrome. J Clin Endocrinol Metab 93:2722-2728.

Stoltz M, Ahlm C, Lundkvist Å and Klingström J (2007): Lambda interferon (IFN-lambda) in serum is decreased in hantavirus-infected patients, and in vitro-established infection is insensitive to treatment with all IFNs and inhibits IFN-gamma-induced nitric oxide production. J Virol 81:8685-8691.

Suh DC, Park JS, Park SK, Lee HK and Chang KH (1995): Pituitary hemorrhage as a complication of hantaviral disease. AJNR Am J Neuroradiol 16:175-178; discussion 179-180.

Tajar A, Forti G, O'Neill TW, Lee DM, Silman AJ, Finn JD, Bartfai G, Boonen S, Casanueva FF, Giwercman A, Han TS, Kula K, Labrie F, Lean MEJ, Pendleton N, Punab M, Vanderschueren D, Huhtaniemi IT, Wu FCW and the EMAS Group (2010):

Characteristics of secondary, primary, and compensated hypogonadism in aging men: evidence from the European male ageing study. J Clin Endocrinol Metab 95:1810-1818.

Takala A, Lähdevirta J, Jansson SE, Vapalahti O, Orpana A, Karonen SL and Repo H (2000): Systemic inflammation in hemorrhagic fever with renal syndrome correlates with hypotension and thrombocytopenia but not with renal injury. J Infect Dis 181:1964-1970.

Temonen M, Vapalahti O, Holthöfer H, Brummer-Korvenkontio M, Vaheri A and Lankinen H (1993): Susceptibility of human cells to Puumala virus infection. J Gen Virol 74 (Pt 3):515-518.

Temonen M, Mustonen J, Helin H, Pasternack A, Vaheri A and Holthöfer H (1996): Cytokines, adhesion molecules, and cellular infiltration in nephropathia epidemica kidneys: an immunohistochemical study. Clin Immunol Immunopathol 78:47-55.

Terajima M, Hayasaka D, Maeda K and Ennis FA (2007): Immunopathogenesis of hantavirus pulmonary syndrome and hemorrhagic fever with renal syndrome: Do CD8+ T cells trigger capillary leakage in viral hemorrhagic fevers? Immunol Lett 113:117-120.

Tersago K, Verhagen R, Servais A, Heyman P, Ducoffre G and Leirs H (2009): Hantavirus disease (nephropathia epidemica) in Belgium: effects of tree seed production and climate. Epidemiol Infect 137:250-256.

Toivanen AL, Valanne L and Tatlisumak T (2002): Acute disseminated encephalomyelitis following nephropathia epidemica. Acta Neurol Scand 105:333-336.

Tsiakalos A, Xynos ID, Sipsas NV and Kaltsas G Pituitary insufficiency after infectious meningitis: a prospective study. J Clin Endocrinol Metab 95:3277-3281.

Tulumovic D, Imamovic G, Mesic E, Hukic M, Tulumovic A, Imamovic A and Zerem E (2010): Comparison of the effects of Puumala and Dobrava viruses on early and long-term renal outcomes in patients with haemorrhagic fever with renal syndrome. Nephrology (Carlton) 15:340-343.

Tuuminen T, Kekäläinen E, Mäkelä S, Ala-Houhala I, Ennis FA, Hedman K, Mustonen J, Vaheri A and Arstila TP (2007): Human CD8+ T cell memory generation in Puumala

hantavirus infection occurs after the acute phase and is associated with boosting of EBV-specific CD8+ memory T cells. J Immunol 179:1988-1995.

Vaheri A, Vapalahti O and Plyusnin A (2008): How to diagnose hantavirus infections and detect them in rodents and insectivores. Rev Med Virol 18:277-288.

Vaheri A, Mills JN, Spiropoulou CF and Hjelle B (2011). Hantaviruses. In: Oxford Textbook of Zoonoses ─ Biology, Clinical Practice and Public Health Control. Eds. Oxford University Press, Oxford U.K.

Valdivieso F, Vial P, Ferres M, Ye C, Goade D, Cuiza A and Hjelle B (2006): Neutralizing antibodies in survivors of Sin Nombre and Andes hantavirus infection. Emerg Infect Dis 12:166-168.

Valtonen M, Kauppila M, Kotilainen P, Lähdevirta J, Svartbäck CM, Kosunen O, Nurminen J, Sarkkinen H and Brummer-Korvenkontio M (1995): Four fatal cases of nephropathia epidemica. Scand J Infect Dis 27:515-517.

Van Epps HL, Schmaljohn CS and Ennis FA (1999): Human memory cytotoxic T-lymphocyte (CTL) responses to Hantaan virus infection: identification of virus-specific and cross-reactive CD8(+) CTL epitopes on nucleocapsid protein. J Virol 73:5301-5308.

Van Epps HL, Terajima M, Mustonen J, Arstila TP, Corey EA, Vaheri A and Ennis FA (2002): Long-lived memory T lymphocyte responses after hantavirus infection. J Exp Med 196:579-588.

Van Loock F, Thomas I, Clement J, Ghoos S and Colson P (1999): A case-control study after a hantavirus infection outbreak in the south of Belgium: who is at risk? Clin Infect Dis 28:834-839.

van Ypersele de Strihou C and Mery JP (1989): Hantavirus-related acute interstitial nephritis in western Europe. Expansion of a world-wide zoonosis. Q J Med 73:941-950.

Wang M, Wang J, Zhu Y, Xu Z, Yang K, Yang A and Jin B (2009): Cellular immune response to Hantaan virus nucleocapsid protein in the acute phase of hemorrhagic fever with renal syndrome: correlation with disease severity. J Infect Dis 199:188-195.

Wang ML, Lai JH, Zhu Y, Zhang HB, Li C, Wang JP, Li YM, Yang AG and Jin BQ (2009): Genetic susceptibility to haemorrhagic fever with renal syndrome caused by Hantaan virus in Chinese Han population. Int J Immunogenet 36:227-229.

Vapalahti K, Paunio M, Brummer-Korvenkontio M, Vaheri A and Vapalahti O (1999): Puumala virus infections in Finland: increased occupational risk for farmers. Am J Epidemiol 149:1142-1151.

Vapalahti K, Virtala AM, Vaheri A and Vapalahti O (2010): Case-control study on Puumala virus infection: smoking is a risk factor. Epidemiol Infect 138:576-584.

Vapalahti O, Kallio-Kokko H, Närvänen A, Julkunen I, Lundkvist Å, Plyusnin A, Lehväslaiho H, Brummer-Korvenkontio M, Vaheri A and Lankinen H (1995): Human B-cell epitopes of Puumala virus nucleocapsid protein, the major antigen in early serological response. J Med Virol 46:293-303.

Vapalahti O, Lundkvist Å, Kukkonen SK, Cheng Y, Gilljam M, Kanerva M, Manni T, Pejcoch M, Niemimaa J, Kaikusalo A, Henttonen H, Vaheri A and Plyusnin A (1996a): Isolation and characterization of Tula virus, a distinct serotype in the genus Hantavirus, family Bunyaviridae. J Gen Virol 77 (Pt 12):3063-3067.

Vapalahti O, Lundkvist Å, Kallio-Kokko H, Paukku K, Julkunen I, Lankinen H and Vaheri A (1996b): Antigenic properties and diagnostic potential of puumala virus nucleocapsid protein expressed in insect cells. J Clin Microbiol 34:119-125.

Vapalahti O, Mustonen J, Lundkvist Å, Henttonen H, Plyusnin A and Vaheri A (2003): Hantavirus infections in Europe. Lancet Infect Dis 3:653-661.

Vasconcelos MI, Lima VP, Iversson LB, Rosa MD, da Rosa AP, da Rosa ES, Pereira LE, Nassar E, Katz G, Matida LH, Zaparoli MA, Ferreira JJ and Peters CJ (1997): Hantavirus pulmonary syndrome in the rural area of Juquitiba, Sao Paulo metropolitan area, Brazil. Rev Inst Med Trop Sao Paulo 39:237-238.

Weidmann M, Schmidt P, Vackova M, Krivanec K, Munclinger P and Hufert FT (2005): Identification of genetic evidence for dobrava virus spillover in rodents by nested reverse transcription (RT)-PCR and TaqMan RT-PCR. J Clin Microbiol 43:808-812.

Vervoort G, Veldman B, Berden JH, Smits P and Wetzels JF (2005): Glomerular hyperfiltration in type 1 diabetes mellitus results from primary changes in proximal tubular sodium handling without changes in volume expansion. Eur J Clin Invest 35:330-336.

Vesely DL, Mastrandrea P, Samson C, Argyelan G and Charvit S (2000): Post-herpes encephalitic anterior pituitary insufficiency with hypothermia and hypotension. Am J Med Sci 320:273-277.

Westman P, Kuismin T, Partanen J and Koskimies S (1993): An HLA-DR typing protocol using group-specific PCR-amplification followed by restriction enzyme digests. Eur J Immunogenet 20:103-109.

Whitworth JA, Stewart PM, Burt D, Atherden SM and Edwards CR (1989): The kidney is the major site of cortisone production in man. Clin Endocrinol (Oxf) 31:355-361.

Williams DG (1998): Mesangiocapillary glomerulonephritis. In: Oxford Textbook of Clinical Nephrology, pp. 591-612. Eds. Davison AM, Cameron JS, Grungeld J-P, Kerr DNS, Ritz E, Winearls CG, Oxford University Press, Oxford.

Wilson AG, di Giovine FS, Blakemore AI and Duff GW (1992): Single base polymorphism in the human tumour necrosis factor alpha (TNF alpha) gene detectable by NcoI restriction of PCR product. Hum Mol Genet 1:353.

Wirta OR and Pasternack AI (1995): Glomerular filtration rate and kidney size in type 2 (non-insulin-dependent) diabetes mellitus. Clin Nephrol 44:1-7.

Wong H, Vivian L, Weiler G and Filler G (2004): Patients with autosomal dominant polycystic kidney disease hyperfiltrate early in their disease. Am J Kidney Dis 43:624-628.

Wu Y, Yang L, Su T, Wang C, Liu G and Li XM (2010): Pathological significance of a panel of urinary biomarkers in patients with drug-induced tubulointerstitial nephritis. Clin J Am Soc Nephrol 5:1954-1959.

Vyssoulis GP, Tousoulis D, Antoniades C, Dimitrakopoulos S, Zervoudaki A and Stefanadis C (2007): Alpha-1 microglobulin as a new inflammatory marker in newly diagnosed hypertensive patients. Am J Hypertens 20:1016-1021.

Wörnle M, Schmid H, Banas B, Merkle M, Henger A, Roeder M, Blattner S, Bock E, Kretzler M, Gröne HJ and Schlöndorff D (2006): Novel role of toll-like receptor 3 in hepatitis C-associated glomerulonephritis. Am J Pathol 168:370-385.

Yanagihara R and Silverman DJ (1990): Experimental infection of human vascular endothelial cells by pathogenic and nonpathogenic hantaviruses. Arch Virol 111:281-286.

Yanagihara R, Gajdusek DC, Gibbs CJ, Jr. and Traub R (1984): Prospect Hill virus: serologic evidence for infection in mammologists. N Engl J Med 310:1325-1326.

Yanagihara R, Amyx HL and Gajdusek DC (1985): Experimental infection with Puumala virus, the etiologic agent of nephropathia epidemica, in bank voles (Clethrionomys glareolus). J Virol 55:34-38.

Yashina L, Mishin V, Zdanovskaya N, Schmaljohn C and Ivanov L (2001): A newly discovered variant of a hantavirus in Apodemus peninsulae, far Eastern Russia. Emerg Infect Dis 7:912-913.

Ye C, Prescott J, Nofchissey R, Goade D and Hjelle B (2004): Neutralizing antibodies and Sin Nombre virus RNA after recovery from hantavirus cardiopulmonary syndrome. Emerg Infect Dis 10:478-482.

Zaki SR, Greer PW, Coffield LM, Goldsmith CS, Nolte KB, Foucar K, Feddersen RM, Zumwalt RE, Miller GL, Khan AS and et al. (1995): Hantavirus pulmonary syndrome. Pathogenesis of an emerging infectious disease. Am J Pathol 146:552-579.

Zeitz PS, Butler JC, Cheek JE, Samuel MC, Childs JE, Shands LA, Turner RE, Voorhees RE, Sarisky J, Rollin PE and et al. (1995): A case-control study of hantavirus pulmonary syndrome during an outbreak in the southwestern United States. J Infect Dis 171:864-870.

Zetterholm SG (1934): Akuta nefriter simulerande akuta bukfall. Svenska Läkartidningen 31:425-429.

Zhu LY, Chi LJ, Wang X and Zhou H (2009): Reduced circulating CD4+CD25+ cell populations in haemorrhagic fever with renal syndrome. Clin Exp Immunol 156:88-96.

Zöller L, Faulde M, Meisel H, Ruh B, Kimmig P, Schelling U, Zeier M, Kulzer P, Becker C, Roggendorf M and Bautz EKF, Krüger DH and Darai G (1995): Seroprevalence of hantavirus antibodies in Germany as determined by a new recombinant enzyme immunoassay. Eur J Clin Microbiol Infect Dis 14:305-313.

Åkerstrom B, Maghzal GJ, Winterbourn CC and Kettle AJ (2007): The lipocalin alpha1-microglobulin has radical scavenging activity. J Biol Chem 282:31493-31503.

ORIGINAL COMMUNICATIONS

Tubular Proteinuria and Glomerular Filtration 6 Years after Puumala Hantavirus-Induced Acute Interstitial Nephritis

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Abstract

Background/Aims: We previously found increased urinary protein excretion, glomerular filtration rate (GFR) and blood pressure in a retrospective analysis of patients with previous nephropathia epidemica (NE). Here, we evaluated the long-term outcome after NE in a prospectively recruited patient group. *Methods:* Proteinuria, GFR and ambulatory 24-hour blood pressure were assessed 4–7 years (mean 6) after acute NE in 37 patients, and these values were compared to those from 38 seronegative controls. *Results:* Six years after NE, the prevalence of elevated urinary α_1-microglobulin excretion was higher in the patients than controls (9/35 vs. 1/38; p = 0.005). The patients also had higher urinary protein excretion (0.17 ± 0.05 vs. 0.14 ± 0.04 g/day; p = 0.006), GFR (119 ± 19 vs. 109 ± 14 ml/min/1.73 m²; p = 0.016) and mean systolic (123 ± 11 vs. 117 ± 9 mm Hg; p = 0.012), nighttime systolic (109 ± 11 vs. 100 ± 9 mm Hg; p = 0.001) and nighttime diastolic blood pressure (70 ± 7 vs. 66 ± 7 mm Hg; p = 0.035) than the controls. *Conclusions:* These results confirm our previous findings of a higher prevalence of tubular proteinuria and increased urinary protein excretion, GFR and systolic blood pressure 6 years after acute NE.

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Introduction

Nephropathia epidemica (NE) is a mild type of hemorrhagic fever with renal syndrome. It is caused by Puumala virus, a member of the hantavirus genus in the Bunyaviridae family that is carried by bank voles (*Myodes glareolus*) [1, 2]. NE is characterized by sudden onset of fever, headache, backache and abdominal pain [3–5]. Proteinuria, hematuria and oliguria followed by polyuria are common clinical findings, while acute tubulointerstitial nephritis is the typical histopathologic lesion on renal biopsy [3, 6]. The clinical picture of the disease varies in severity, and complete recovery is the usual outcome [3–5].

Previously, we performed a retrospective analysis of 46 patients 5 years after acute NE and found higher urinary protein excretion and increased glomerular filtra-

tion rate (GFR), filtration fraction (FF) and systolic blood pressure in the patients than in healthy control subjects [7]. Recently, we were able to re-examine 36 patients from the same group 5 years later, i.e. 10 years after acute NE. At that stage the differences between the patients and controls with regard to proteinuria, GFR, FF and blood pressure were no longer present, which supports the view that the long-term prognosis of NE is favorable [8].

In the present study, we evaluated the reliability of the results of our previous retrospective investigation [7] and examined urinary protein excretion, renal function and ambulatory blood pressure (ABP) in a new prospectively recruited group of patients 4–7 years (mean 6) after acute NE. The results were compared with those of the same seronegative controls as in our previous study [7], in order to avoid the influence of possible variations between different control populations.

Methods

Study Population and Protocol

The patient group consisted of 43 consecutive patients hospitalized due to serologically verified NE at Tampere University Hospital [9], Finland, during the years 1997–1999. They were prospectively collected for this longitudinal study. Six of these 43 patients were excluded from the present study; 4 of them had hypertension before acute NE, 1 patient had diabetic nephropathy and 1 had mesangial glomerulonephritis. Thirty-seven patients (29 males and 8 females) aged 29–70 years (mean 49) were examined as outpatients 4–7 years (median 6) after acute NE in 2004. One patient had suffered from Henoch-Schöenlein purpura in childhood and 1 patient had been treated for renal tuberculosis in the 1960s. Neither of them showed any findings of chronic renal disease following these conditions. Prior to the episode of acute NE, the other chronic diseases in 7 of the patients were hypothyroidism (in 3 patients) and celiac disease, coronary heart disease, bronchial asthma and ankylosing spondylarthritis (each in 1 patient). The patient group was compared with the same group of 38 healthy, Puumala virus-seronegative controls (22 males and 16 females), aged from 27 to 64 years (mean 44), that was used in our previous study [7]. One of the controls had hypothyroidism. All 4 subjects with hypothyroidism were euthyreotic with thyroxin substitution.

All subjects gave informed consent before participation, and the study was approved by the Ethics Committee of Tampere University Hospital.

Laboratory Specimens and Electrocardiogram

Blood specimens were obtained in the morning after a minimum 12-hour fast. In the studies that took place from 1997 to 1999, serum creatinine, urea, triglycerides, blood glucose and total, high-density and low-density lipoprotein cholesterol were determined by Vitros (Johnson & Johnson, Rochester, N.Y., USA), and the blood cell count was evaluated by Technicon H3 (Bayer Diagnostics, Elkhart, Ind., USA). In 2004, the same laboratory parameters were analyzed by Cobas Integra 700/800 and Bayer Advia 120, respectively. Serum creatinine concentrations showed 10% lower values in 2004 than during the years 1997–1999 due to the above change in the determination method. Fasting blood glucose was determined from whole blood until the year 2003, after which the analysis was performed in plasma, thus producing 12% higher values. In this study, the results of serum creatinine concentrations from the years 1997–1999 were multiplied by the coefficient 0.9, and the results of fasting plasma glucose before the year 2003 were multiplied by the coefficient 1.12. The limits for impaired fasting glucose (IFG) were 6.1–6.9 mmol/l, according to the guideline of the European Diabetes Epidemiology Group [10].

Nighttime, daytime and 24-hour urinary protein excretion was measured by the pyrogallol red molybdate method (Olli C, Kone Instruments, Helsinki, Finland) in the 1990s and by Cobas Integra 700/800 in 2004. The overnight urinary excretion of α_1-microglobulin, albumin and immunoglobulin G (IgG) was measured by nephelometry (Behring Nephelometer II Analyzer, Behringwerke AG, Marburg, Germany). Values for overnight urinary excretion of α_1-microglobulin ≥ 7 μg/min, albumin ≥ 11 μg/min and IgG ≥ 5 μg/min were considered abnormal, based on the reference values for healthy subjects from our laboratory. The spot samples of morning urine were collected after a minimum of 12 h of fasting and analyzed for osmolality, erythrocytes, leukocytes, albumin, nitrite, glucose, pH and ketones. Urine osmolality was measured by the Advanced Cryomatic™ Osmometer (Advanced Instruments Inc., Needham Heights, Mass., USA). Urine for the measurement of proteinuria was not collected for 3 patients.

Electrocardiograms (ECGs) were evaluated according to the Minnesota codes. Left ventricular mass was evaluated by the sum of the height of the S wave in lead V1 and the R wave in lead V5 of a 12-lead resting ECG. Body mass index was calculated by dividing weight (in kilograms) by the square of height (in square meters).

Determination of Renal Function

GFR was determined by a single-injection method as the plasma clearance of ^{51}Cr-EDTA after a light meal and expressed in values normalized for body surface area. Effective renal plasma flow (ERPF) was estimated by the clearance of ^{131}I-hippurate. The FF was calculated as the quotient of GFR and ERPF. Based on studies in healthy subjects, increased GFR was defined as a GFR ≥ 130 ml/min/1.73 m^2 [11].

Blood Pressure Monitoring

The 24-hour ABP was measured with a fully automatic recorder. The cutoff point for hypertension was a 24-hour mean ABP $\geq 133/82$ mm Hg, according to the results of a large population study [12]. One control subject and 10 patients either refused ABP measurement or had missing measurements due to technical failure.

Statistical Analysis

To describe the data, means and standard deviations (SDs) are given for normally distributed variables and medians and ranges for skew-distributed continuous variables. Groups were compared using independent-sample t tests for normally distributed variables and Mann-Whitney U tests for skewed variables. Cate-

Table 1. Clinical and basic laboratory findings in patients 6 years after acute NE and in controls

	Patients (n = 37)	Controls (n = 38)	p value
Males/females[a]	29/8 (78)	22/16 (58)	NS
Age, years	49 ± 11	44 ± 10	0.042
Body mass index	26 (20–47)	25 (21–34)	NS
Blood hemoglobin, g/l	144 ± 9	141 ± 11	NS
Serum creatinine[b], μmol/l	70 ± 12	73 ± 11	NS
Serum cholesterol, mmol/l	5.5 (4.4–8.8)	5.2 (4.0–7.8)	0.039
Serum LDL cholesterol, mmol/l	3.5 (2.4–5.3)	3.2 (2.2–4.7)	NS
Serum triglycerides, mmol/l	1.12 (0.46–5.11)	1.38 (0.49–3.57)	NS
Fasting blood glucose[b], mmol/l	5.7 ± 0.6	5.1 ± 0.5	<0.001
Urine osmolality, mOsm/kg	776 ± 175	749 ± 221	NS

Means ± SD are given for normally distributed variables and medians (range) for skew-distributed variables. LDL = Low-density lipoprotein; NS = not significant.
[a] Figures in parentheses represent the percentage of males.
[b] Determination methods for serum creatinine and fasting blood glucose changed during the study (see Methods).

gorical data were analyzed by χ^2 test or by Fisher's exact test. Correlations were analyzed by Pearson's correlation coefficient. Skew-distributed urinary excretion of protein was log transformed before analysis. Binary logistic regression analysis was carried out to determine whether the differences in proteinuria, GFR and mean systolic, nighttime systolic or nighttime diastolic blood pressure between the groups could be ascribed to the differences in age and gender distribution between the groups. Odds ratios (ORs) and their 95% confidence intervals (CIs) were given. All tests were two-sided, and analyses were performed with SPSS for Windows, version 13 (SPSS Inc., Chicago, Ill., USA).

Results

Clinical and Laboratory Data

The characteristics of the study population and basic laboratory values are shown in table 1. The patients were on average 5 years older than the controls, and their fasting blood glucose and serum low-density lipoprotein cholesterol levels were slightly higher than in controls. Medication for type 2 diabetes had been initiated for 1 male patient after acute NE, but he showed no evidence of albuminuria during this study. One female patient had fasting blood glucose of 7.1 mmol/l and her hemoglobin A1 concentration was 6.4%, pointing to type 2 diabetes. Recurrent microscopic hematuria, in the absence of proteinuria, was also detected after NE. Her clinical, laboratory and radiological examinations showed normal blood pressure, renal function and renal ultrasound. Moreover, there were no specific findings on cystoscopy, while uri-

nary cytology showed class II cells with atypical degenerative changes. Seven out of 37 patients (19%) had IFG, while none of the 38 controls had abnormal fasting blood glucose (p = 0.005, Fisher's exact test). Altogether, 9 of the 37 patients (24%) had IFG or type 2 diabetes.

One patient had right bundle branch block on the ECG, but no pathological Q waves or ST-T wave alterations were recorded. The sum of the S wave in lead V1 and R wave in lead V5 did not differ between the patients and controls (24 and 23 mm, respectively; p = 0.482, independent-samples t test).

Proteinuria and Renal Function

Table 2 and figure 1 show proteinuria (fig. 1a) and renal function (fig. 1b) in patients 6 years after acute NE compared with seronegative controls. The urinary protein excretion was higher in patients than in controls, and after adjusting for age and sex, the difference still remained statistically highly significant. A positive correlation between proteinuria and GFR was detected in the patients (r = 0.495, p = 0.003, Pearson's correlation) but not in the controls (r = 0.255, p = 0.123), while proteinuria did not correlate with age or systolic or diastolic blood pressure in either group (data not shown). In addition, the prevalence of elevated urinary excretion of α_1-microglobulin was higher among the patients than the controls (9/35 vs. 1/38; p = 0.005, Fisher's exact test), but there was no difference between the groups in the overnight excretion of albumin or IgG (data not shown).
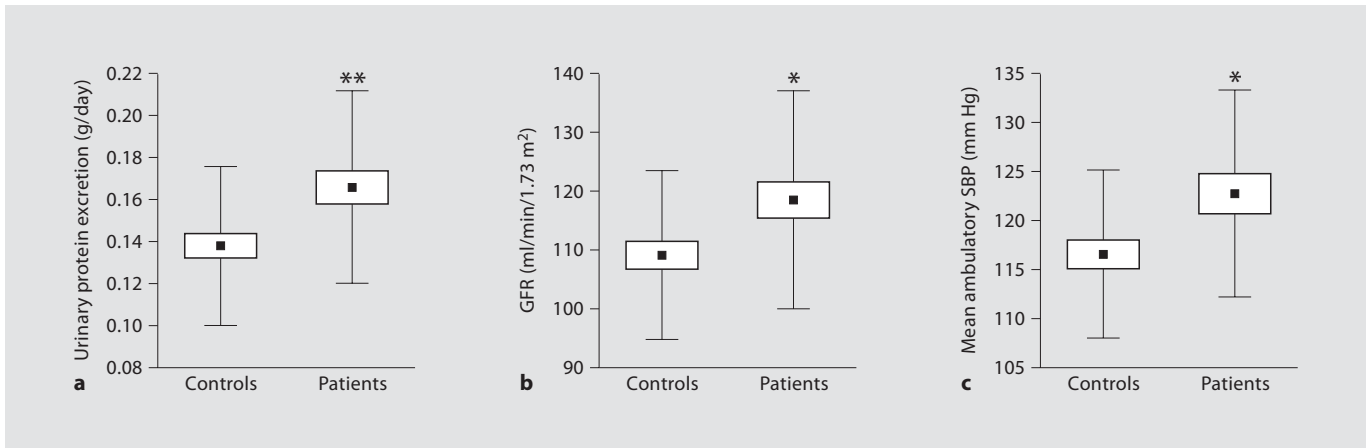
Fig. 1. Urinary protein excretion (**a**), GFR (**b**) and mean ambulatory systolic blood pressure (SBP) (**c**) in controls (n = 38) and prospectively recruited patients (n = 36) 6 years after acute NE. Variables are depicted as means (■), standard errors of the mean (boxes) and SD (whiskers). * p < 0.05; ** p < 0.01.

Table 2. Proteinuria, renal function and ABP in patients 6 years after acute NE and in controls

	Patients (n = 27–37)	Controls (n = 37–38)	p value
Proteinuria, g/day	0.17 ± 0.05	0.14 ± 0.04	0.006
GFR, ml/min/1.73 m²	119 ± 19	109 ± 14	0.016
ERPF, ml/min/1.73 m²	646 ± 116	627 ± 105	NS
FF, %	19 ± 3	18 ± 3	NS
Mean 24-hour BP, mm Hg			
SBP	123 ± 11	117 ± 9	0.012
DBP	78 ± 6	76 ± 7	NS
Mean daytime BP, mm Hg			
SBP	125 ± 12	121 ± 9	NS
DBP	80 ± 7	79 ± 7	NS
Mean nighttime BP, mm Hg			
SBP	109 ± 11	100 ± 9	0.001
DBP	70 ± 7	66 ± 7	0.035

BP = Blood pressure; SBP = systolic blood pressure; DBP = diastolic blood pressure; NS = not significant.

The mean GFR was higher in patients than in controls, but there was no difference in ERPF or FF between the groups (table 2, fig. 1b). After adjusting for age and sex, the difference in GFR remained statistically significant (OR 1.047, 95% CI 1.01–1.09; p = 0.010). Increased GFR (≥130 ml/min/1.73 m²) was found in 10 of the 37 patients (27%) compared to 4 of the 38 controls (11%) (p = 0.067, χ^2 test). GFR correlated inversely with age in the patient group (r = –0.512, p = 0.001, Pearson's correlation) but not in controls (r = 0.008, p = 0.963). Furthermore, GFR showed no correlation with blood pressure or fasting blood glucose in either group (data not shown). The mean GFR was 118 ± 15 ml/min/1.73 m² in patients with diabetes or IFG and 119 ± 20 ml/min/1.73 m² in patients with normal fasting blood glucose (p = 0.194, independent-samples t test). Furthermore, increased GFR was found in 3 of the 9 patients with diabetes or IFG compared to 7 of the 28 patients with normal fasting blood glucose (p = 0.679, Fisher's exact test). The clinical severity of acute NE, as indicated by the highest serum creatinine concentration measured during the hospital stay, had no relation to proteinuria or GFR 6 years later (data not shown).

Blood Pressure

ABP monitoring was successfully completed in 27 patients and 37 controls. The patients had higher mean (fig. 1c) and nighttime systolic blood pressure and nighttime diastolic blood pressure than the controls (table 2).

After adjusting for age and sex, the difference between the groups with regard to mean and nighttime systolic blood pressure remained (OR 1.07, 95% CI 1.00–1.14, p = 0.038, and OR 1.08, 95% CI 1.01–1.14, p = 0.019, respectively). In the whole group of study subjects, neither the mean, daytime nor nighttime systolic and diastolic blood pressure correlated with age (data not shown). The highest serum creatinine measured during acute NE had no relation to the results of ABP measurement 6 years later (data not shown).

Antihypertensive medication had been initiated in 5 of the patients 1–6 years after acute NE, and all continued their medication during the examinations. The classes of the medications were β-adrenoceptor blockers, calcium channel blockers, angiotensin-converting enzyme inhibitors and angiotensin II receptor antagonists. None of the controls were on antihypertensive medication during the study. It is noteworthy that differences in mean systolic and nighttime systolic blood pressure between the groups remained significant even if the subjects using antihypertensive medication were excluded from the statistical analysis.

Discussion

The long-term prognosis of Puumala hantavirus-induced acute tubulointerstitial nephritis is considered to be favorable. However, this longitudinal study with a prospectively recruited patient group confirmed our previous findings that 4–7 years after acute NE, patients have higher urinary protein excretion, GFR and ambulatory systolic blood pressure than healthy controls [7].

As also previously described, this study showed increased urinary protein excretion and higher prevalence of abnormal α_1-microglobulin excretion in patients than in controls several years after NE [7, 13]. In a recent retrospective analysis of 30 Croatian patients, performed 3–6 years after acute hemorrhagic fever with renal syndrome, 6 of the 23 patients (26%) had increased excretion of β_2-microglobulin, while proteinuria exceeding 150 mg/day was detected in 11 of the 23 patients (48%) [14]. The pathophysiological mechanism of mild proteinuria several years after acute hemorrhagic fever with renal syndrome is obscure. However, in 1978, Lähdevirta et al. [15] from Finland published a study of renal biopsies from 9 patients 4–5 years after the episode of acute NE. The samples showed slight residual interstitial fibrosis and occasionally atrophic tubuli, tubular casts, increased numbers of hyalinised glomeruli and minor changes in other glomeruli. Two specimens also showed fibrotic scars in the cortex.

In this study, the patients who presented with higher GFR than the controls 6 years after acute NE were older than the controls, who were age-matched with the previous patient cohort [7]. Usually GFR declines with age [16], and also in the present study, GFR correlated inversely with age in the patient group. This indicates that age cannot explain the difference in GFR between the groups. The mechanism underlying increased glomerular filtra-tion after acute interstitial nephritis is not known at present. In early diabetic nephropathy, glomerular hyperfiltration is a typical feature, and similar findings have been reported in essential hypertension and polycystic kidney disease [17–20]. Furthermore, in a recent report on stage I hypertensive patients, glomerular hyperfiltration was shown to predict the development of microalbuminuria [21]. In the present investigation, increased GFR in patients might have been related to the higher frequency of IFG in patients than in controls. However, the GFR of patients with diabetes or IFG compared with the patients with normal fasting blood glucose was essentially the same. Neither did we find any correlation between the GFR and fasting blood glucose levels in this study. Higher GFR could also have been a consequence of the higher systolic blood pressure in the patient group, although the fact that GFR showed no correlation with blood pressure is in conflict with this view.

Our patients had higher ambulatory systolic blood pressure than the controls 4–7 years after acute NE, as in our previous study [7]. Furthermore, antihypertensive medication had been started in 5 patients during the follow-up, and all of them continued their medication during the study. If the subjects treated with antihypertensive compounds were excluded from the analyses, the outcome of the analysis of ABP results remained the same, i.e. the patients still showed increased systolic blood pressure. Furthermore, we did not find any significant correlation between age and blood pressure, and the difference in blood pressure between the groups remained after adjusting for age and sex.

We recently re-examined 36 patients from our previous retrospective analysis of a patient cohort 10 years after acute NE [8]. The results showed no difference in proteinuria, GFR or FF between the patients and the controls. As in all long-term clinical follow-up studies, there were some dropouts. As the dropouts did not differ essentially from the study population, it seems safe to conclude that the 10-year prognosis of NE is favorable [22]. Nevertheless, the possibility remains that NE may predispose some patients to the development of hypertension.

In conclusion, in a new prospectively recruited population of patients with prior NE, we were able to confirm our previous findings of higher prevalence of tubular proteinuria, increased GFR and higher systolic blood pressure 6 years after acute Puumala hantavirus-induced nephritis compared with a healthy control group. The underlying mechanisms of the tubular proteinuria and the increased glomerular filtration remain unclear.

References

1 Vapalahti O, Mustonen J, Lundkvist Å, Henttonen O, Plyusnin A, Vaheri A: Hantavirus infections in Europe. Lancet Infect Dis 2003;3:653–661.
2 Muranyi W, Bahr U, Zeier M, van der Woude FJ: Hantavirus infection. J Am Soc Nephrol 2005;16:3669–3679.
3 Lähdevirta J: Nephropathia epidemica in Finland. A clinical histological and epidemiological study. Ann Clin Res 1971;3:1–54.
4 Mustonen J, Brummer-Korvenkontio M, Hedman K, Pasternack A, Pietilä K, Vaheri A: Nephropathia epidemica in Finland: a retrospective study of 126 cases. Scand J Infect Dis 1994;26:7–13.
5 Settergren B: Clinical aspects of nephropathia epidemica (Puumala virus infection) in Europe: a review. Scand J Infect Dis 2000; 32:125–132.
6 Mustonen J, Helin H, Pietilä K, Brummer-Korvenkontio M, Hedman K, Vaheri A, Pasternack A: Renal biopsy findings and clinicopathologic correlations in nephropathia epidemica. Clin Nephrol 1994;41:121–126.
7 Mäkelä S, Ala-Houhala I, Mustonen J, Koivisto AM, Kouri T, Turjanmaa V, Vapalahti O, Vaheri A, Pasternack A: Renal function and blood pressure five years after puumala virus-induced nephropathy. Kidney Int 2000;58:1711–1718.
8 Miettinen MH, Mäkelä SM, Ala-Houhala IO, Huhtala HS, Kööbi T, Vaheri AI, Pasternack AI, Pörsti IH, Mustonen JT: Ten-year prognosis of Puumala hantavirus-induced acute interstitial nephritis. Kidney Int 2006; 69:2043–2048.
9 Vapalahti O, Lundkvist Å, Kallio-Kokko H, Paukku K, Julkunen I, Lankinen H, Vaheri A: Antigenic properties and diagnostic potential of puumala virus nucleocapsid protein expressed in insect cells. J Clin Microbiol 1996;34:119–125.
10 Forouhi NG, Balkau B, Borch-Jonsen K, Dekker J, Glumer C, Qiao Q, Spijkerman A, Stolk R, Tabac A, Wareham NJ: The threshold for diagnosing impaired fasting glucose: a position statement by the European Diabetes Epidemiology Group. Diabetologia 2006; 49:822–827.
11 Vervoort G, Veldman B, Berden JHM, Smits P, Wetzels JFM: Glomerular hyperfiltration in type 1 diabetes mellitus results from primary changes in proximal tubular sodium handling without changes in volume expansion. Eur J Clin Invest 2005;35:330–336.
12 Staessen J, O'Brien E, Amery A, Atkins N, Baumgart P, De Cort P, Degaute JP, Dolenc P, De Gaudemaris R, Enstrom I, et al: Ambulatory blood pressure in normotensive and hypertensive subjects: results from an international database. J Hypertens 1994;12(suppl): S1–S12.
13 Ala-Houhala I, Koskinen M, Ahola T, Harmoinen A, Kouri T, Laurila K, Mustonen J, Pasternack A: Increased glomerular permeability in patients with nephropathia epidemica caused by Puumala hantavirus. Nephrol Dial Transplant 2002;17:246–252.
14 Ledina D, Bradaric N, Ivic I, Marasovic D, Radovic D, Ostojic-Bakotin V, Prgomet S: Is permanent renal function damage possible after hemorrhagic fever with renal syndrome? (in Croatian). Acta Med Croatica 2003;57:365–368.
15 Lähdevirta J, Collan Y, Jokinen EJ, Hiltunen R: Renal sequelae to nephropathia epidemica. Acta Pathol Microbiol Scand [A] 1978;86: 265–271.
16 Brenner BM, Rector FC (eds): The Kidney. Philadelphia, WB Saunders, 2004.
17 Mogensen CE: Microalbuminuria as a predictor of clinical diabetic nephropathy. Kidney Int 1987;31:673–689.
18 Wirta OR, Pasternack AI: Glomerular filtration rate and kidney size in type 2 (non-insulin-dependent) diabetes mellitus. Clin Nephrol 1995;44:1–7.
19 Schmieder RE, Veelken R, Schobel H, Dominiak P, Mann JF, Luft FC: Glomerular hyperfiltration during sympathetic nervous system activation in early essential hypertension. J Am Soc Nephrol 1997;8:893–900.
20 Wong H, Vivian L, Weiler G, Filler G: Patients with autosomal dominant polycystic kidney disease hyperfiltrate early in their disease. Am J Kidney Dis 2004;43:624–628.
21 Palatini P, Mormino P, Dorifatti F, Santonastaso M, Mos L, De Toni R, Winnicki M, Dal Follo M, Biasion T, Garavelli G, Pessina AC: Glomerular hyperfiltration predicts the development of microalbuminuria in stage 1 hypertension: the HARVEST. Kidney Int 2006;70:578–584.
22 Rippe B: Is there an increased long-term risk of hypertension and renal impairment after Puumala virus-induced nephropathy? Kidney Int 2006;69:1930–1931.

nephron
Clinical Practice

The Severity of Acute Puumala Hantavirus Infection Does Not Predict the Long-Term Outcome of Patients

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Abstract

Background/Aims: We have found greater urinary protein excretion and higher glomerular filtration rate (GFR) and blood pressure in patients 6 years after acute nephropathia epidemica (NE) compared with seronegative controls. The present aim was to establish whether the long-term outcome is determined by the severity of acute illness. ***Methods:*** Serial plasma interleukin-6 (IL-6), tumour necrosis factor-α (TNF-α), creatinine, C-reactive protein, blood cell count as well as 24-hour urinary protein and overnight α_1-microglobulin and albumin excretions were measured in 37 patients with acute NE. Human leucocyte antigen (HLA)-B, HLA-DRB1, TNF-α(–308) and IL-6(–174) alleles were also analyzed. After 6 years, GFR, blood pressure and urinary protein excretion were examined. ***Results:*** There were no associations between the clinical severity of acute NE or the genetic factors determined and the increased GFR, hypertension or 24-hour urinary protein excretion observed 6 years later. The degree of inflammation during the acute phase was higher in patients who had increased urinary excretion of α_1-microglobulin 6 years later compared with those with no α_1-microglobulin excretion. ***Conclusion:*** Neither the severity of acute NE nor the host genetic factors determined the predicted renal function, blood pressure or 24-hour urinary protein excretion 6 years later.

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Introduction

Puumala hantavirus causes a mild type of hemorrhagic fever with renal syndrome, a condition also known as nephropathia epidemica (NE). An average of 1,000–3,000 serological diagnoses have been made annually in Finland during recent years, but the seroprevalence is 5%, implying that most infections remain undiagnosed [1]. Typical symptoms are acute onset of fever, headache, backache and abdominal pain [2, 3]. Renal involvement results in proteinuria, microscopic haematuria and acute renal failure with oliguria followed by polyuria [3]. The

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characteristic renal histopathological lesion is acute tubulointerstitial nephritis [4]. The clinical severity varies considerably, but complete recovery is the rule [2, 3, 5].

The long-term prognosis of NE seems to be favourable [2, 5, 6]. However, we have previously found in 2 independent groups of patients that 5–6 years after acute NE patients had higher systolic blood pressure and a glomerular filtration rate (GFR), and greater urinary protein excretion compared with healthy seronegative controls [7, 8]. As far as we know, no studies have addressed the effect of clinical severity of the acute disease on the possible long-term consequences several years after NE.

Several cytokines are secreted in response to a hantavirus infection [9]. Significantly elevated plasma levels of tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6), IL-2 and interferon-γ (IFN-γ) have been detected during the acute phase of NE [10, 11]. Our study of cytokine levels during acute NE showed that especially urinary excretion of IL-6 was markedly increased [11]. The IL-6(–174) G/C polymorphism has been shown to be associated with plasma IL-6 levels, higher levels being found in homozygotes for allele G and G/C heterozygotes in healthy subjects [12]. Furthermore, there is evidence of genetic predisposition to the severity of NE, as patients with human leucocyte antigen (HLA)-B8, -DR3, and -DQ2 alleles have proved to be at a higher risk of severe disease [13, 14], while patients with the HLA-B27 allele have presented with mild disease [15].

The aim of the present study was to establish whether the severity of the disease or inflammation during the acute phase of NE, or host genetic factors, could predict the clinical outcome 6 years later.

Methods

Study Design and Participants
Originally, this study involved 70 prospectively collected consecutive patients who were admitted to Tampere University Hospital, Finland, due to serologically verified NE [16] during the years 1997–1999. In 2004, 4–7 (median 6) years after acute NE, 43 out of these 70 patients attended a scheduled follow-up examination. The clinical characteristics of these patients have recently been described in detail [8]. Six of the 43 patients were now excluded; 4 patients had had hypertension before acute NE, 1 had diabetic nephropathy and 1 mesangial glomerulonephritis. The study population thus comprised 37 patients (29 males and 8 females) aged from 29 to 70 years (mean 49). All subjects gave informed consent before participation, and the study was approved by the Ethics Committee of Tampere University Hospital.

Blood samples for analysis of plasma IL-6, TNF-α, C-reactive protein (CRP), serum creatinine, and blood cell count, as well as 24-hour urinary protein and overnight α$_1$-microglobulin, albumin, IL-6 and TNF-α excretions were collected on 3 consecutive mornings after hospital admission. The highest and the lowest values of the various variables measured during hospitalization were designated as maximum and minimum.

Since the degree of inflammation can be evaluated by several parameters, we determined an inflammation score to describe it. The maximum values of acute-phase plasma CRP, IL-6 and blood leucocytes were categorized into 2 groups by dividing each variable from its median value (lower than median/higher than median). The inflammation score was then calculated by counting up the number of variables whose values were over the median, and grouped them as follows: combined median values ≤1 (group 1) and ≥2 (group 2).

Laboratory Specimens
Plasma CRP levels were analyzed by a Hitachi 705E analyzer from 1997 to 1998 and thereafter by the Roche Diagnostics CRP method using a Cobas Integra analyzer. Blood cell counts were conducted on haematological cell counters by Bayer. From 1997 to 1999 serum creatinine was determined by Vitros (Johnson & Johnson, Rochester, N.Y., USA), and in 2004 by a Cobas Integra analyzer. Serum creatinine concentrations showed 10% lower values in 2004 than during the years from 1990 to 1999, this by reason of the above change of determination method. For this study, results for serum creatinine concentrations during the years 1990–1999 were multiplied by the coefficient 0.9.

Plasma and urinary TNF-α and IL-6 concentrations were determined as previously described [11]. The detection limits for the assays were 0.4 pg/ml for IL-6 and 1.4 pg/ml for TNF-α. In calculations, a concentration less than the detection limit was regarded as the detection limit for the assay in question. The highest plasma or urinary cytokine concentration measured during the first 3 days of hospitalization was designated the maximum cytokine concentration and used in calculations. The analyses of alleles HLA-B8, -B27 and -DR3, and the analysis of TNF-α and IL-6 gene polymorphism were performed as described elsewhere [17–19].

The 24-hour urinary protein excretion was measured by the pyrogallol red molybdate method (Olli C.; Kone Instruments, Helsinki, Finland) in the 1990s and by the Cobas Integra analyzer in 2004. The overnight urinary excretion of α$_1$-microglobulin and albumin were measured by nephelometry (Behring Nephelometer II analyzer, Behringwerke AG, Marburg, Germany). Overnight urinary excretion of α$_1$-microglobulin ≥7 µg/min and albumin ≥11 µg/min were considered abnormal based on healthy reference material in our laboratory. The limit for increased 24-hour protein excretion was ≥0.22 g.

Determination of Renal Function
GFR was determined by the single-injection method as a plasma clearance of ^{51}Cr-EDTA after a light meal, and expressed in values normalized for body surface area. Based on studies in healthy subjects, increased glomerular filtration was defined as a GFR ≥130 ml/min/1.73 m^2 [20].

Blood Pressure Monitoring
Six years after NE, the 24-hour ambulatory blood pressure (ABP) was measured with a fully automatic recorder (Novacor Diasys Integra; Novacor SA, Rueil-Malmaison, France). The cutoff point for hypertension was a 24-hour mean ABP ≥133/82 mm Hg as established in a large population study [21]. Office blood

Table 1. Clinical and laboratory variables during the acute phase of NE in those patients who had increased overnight urinary excretion of α_1-microglobulin, increased GFR or hypertension 6 years later compared with those who did not

Acute phase of NE	6 years after acute NE								
	urinary α_1-microglobulin (n = 35)			GFR (n = 37)			blood pressure (n = 37)		
	increased (n = 9)	normal (n = 26)	p	increased (n = 10)	normal (n = 27)	p	hypertensives (n = 10)	normotensives (n = 27)	p
B-Hcr$_{min}$, %	37.3 ± 4.0	35.7 ± 4.1	0.291	36.3 ± 2.4	36.2 ± 4.5	0.939	36.5 ± 2.9	36.1 ± 4.4	0.796
B-Throm$_{min}$, 10^9/l	52 ± 26	68 ± 32	0.190	60 ± 32	64 ± 32	0.770	75 ± 28	59 ± 33	0.192
B-Leuk$_{max}$, 10^9/l	8.6 (7.2–20.4)	11.2 (5.7–22.6)	0.838	11.9 (7.1–22.6)	10.3 (5.7–20.4)	0.302	12.3 (7.1–16.6)	10.2 (5.7–22.6)	0.257
S-CRP$_{max}$, mg/l	84 (50–213)	64 (20–175)	0.128	80 (20–175)	72 (22–213)	0.656	100 (22–213)	69 (20–157)	0.098
S-Crea$_{max}$, μmol/l	242 (81–653)	241 (70–878)	0.725	285 (78–626)	192 (70–878)	0.906	321 (70–878)	187 (77–749)	0.335
dU-Protein$_{max}$, g/day	1.78 (0.71–17.78)	1.68 (0.30–10.00)	0.697	1.74 (0.14–10.00)	1.66 (0.30–17.78)	0.801	1.75 (0.30–4.85)	1.66 (0.14–17.78)	0.555
cU-Alb$_{max}$, μg/min	987 (211–6,246)	724 (14–5,793)	0.753	895 (4–5,642)	708 (14–6,246)	0.578	997 (14–2,980)	709 (4–6,246)	0.880
cU-α_1m$_{max}$, μg/min	37 (11–46)	27 (7–209)	0.725	24 (7–209)	29 (11–137)	0.511	29 (11–209)	26 (7–137)	0.371
Hospital stay, days	8 ± 3	7 ± 2	0.468	8 ± 2	7 ± 2	0.634	8 ± 2	7 ± 2	0.305

Means are given for normally distributed variables and median (range) for skew-distributed variables. Groups were compared by independent samples t test (normally distributed variables) or Mann-Whitney U test (skew-distributed variables). B-Hcr$_{min}$ = Lowest blood hematocrit; B-Throm$_{min}$ = lowest blood platelet count; B-Leuk$_{max}$ = highest blood leukocyte count; S-CRP$_{max}$ = highest serum C-reactive protein concentration; S-Crea$_{max}$ = highest serum creatinine; dU-Protein$_{max}$ = highest daily urinary protein excretion; cU-Alb$_{max}$ = highest overnight urinary albumin excretion; cU-α_1m$_{max}$ = highest overnight urinary α_1-microglobulin.

pressure was also measured, the cutoff point for hypertension being according to the World Health Organization/International Society of Hypertension guidelines $\geq$140/90 mm Hg [22]. We defined patients as hypertensive if they were on antihypertensive therapy, had a mean ABP $\geq$133/82 mm Hg, or office blood pressure $\geq$140/90 mm Hg. Office blood pressure was used to define hypertension only if ABP monitoring had not been conducted. Ten out of the 37 patients (27%) either refused ABP measurement or it failed for technical reasons.

Statistical Methods
To describe the data, means and standard deviations (SD) are given for normally distributed variables, and medians and ranges for skew-distributed continuous variables. Patients were divided into 2 groups as having or not having elevated excretion of α_1-microglobulin, increased GFR or hypertension, respectively. The groups were compared using the independent samples t test for normally distributed variables and the Mann-Whitney U test for skewed variables. Categorical data were analyzed by χ^2 test or Fisher's exact test. All tests were 2-sided and analyses were performed with the SPSS for Windows version 14.0 (SPSS Inc., Chicago, Ill., USA).

Results

Clinical and Laboratory Findings 6 Years after Acute NE
Six years after NE, the mean serum creatinine level in the patients was 70 ± 12 μmol/l and 5/37 (14%) patients were on anti-hypertensive medication. Nine out of 35

(26%) patients had elevated urinary excretion of α_1-microglobulin. This was not related to the systolic or diastolic blood pressure (data not shown). Increased 24-hour urinary protein excretion was detected in 4/33 (12%) patients and albuminuria in 2/35 (6%). Ten out of 37 patients (27%) had GFR $\geq$130 ml/min/1.73 m^2 and 10/37 (27%) had hypertension according to our criteria.

The Association of the Clinical Findings during the Acute Phase of NE with the Outcome after 6 Years
The relationships between the clinical and laboratory variables describing the clinical severity of acute NE and the prevalence of urinary excretion of α_1-microglobulin, increased GFR and hypertension 6 years later are shown in table 1. There were no differences in any acute-phase variable studied between those patients who suffered from sequelae 6 years later compared to those who did not. Further, acute-phase findings did not differ between patients showing increased overnight urinary excretion of albumin or 24-hour protein excretion after 6 years (data not shown).

Three out of 37 (8%) patients needed dialysis treatment during the acute phase. One of these patients showed elevated urinary excretion of α_1-microglobulin and hypertension 6 years after NE. GFR ranged from 100 to 121 ml/min/1.73 m^2 6 years after NE in those patients who had required dialysis treatment during the acute illness.

Fig. 1. Highest plasma IL-6 (**a**) and TNF-α (**b**) concentration during acute NE in patients who had increased excretion of α$_1$-microglobulin 6 years later compared with those who did not. Variables are depicted as medians (–), quartiles (boxes), and minimum and maximum values (whiskers) and outliers. * $p < 0.05$.
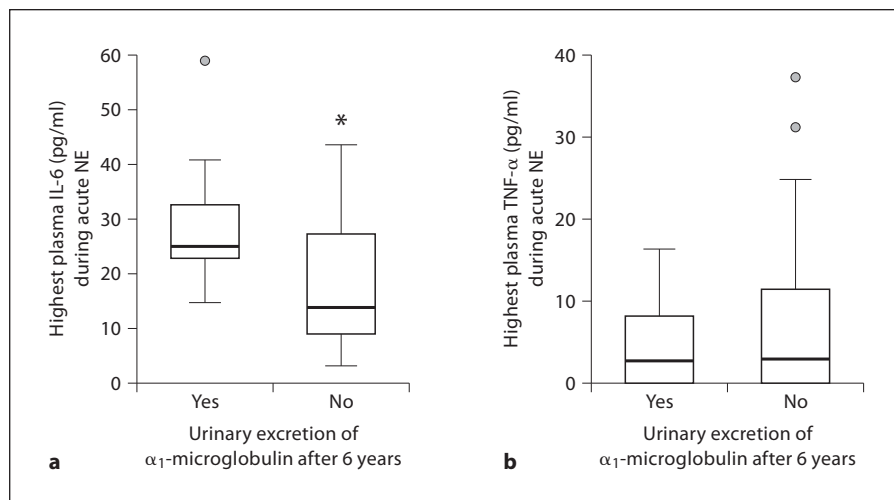
Host Genetic Factors and Clinical Findings 6 Years Later

Eight out of 32 (25%) patients had both HLA-B8 and -DR3 alleles and 3 out of 31 (10%) had the HLA-B27 allele. Thirteen out of 37 (37%) patients were TNF(–308) allele A carriers and 76% (28/37) were IL-6(–174) allele G carriers. The HLA-B8-DR3 haplotype or HLA-B27 allele, the carriage of TNF(–308) allele A or IL-6(–174) allele G was not related to renal function, urinary protein excretion or hypertension 6 years after acute NE (data not shown).

Plasma Levels and Urinary Excretion of Cytokines during the Acute Phase of NE Compared with the Clinical Findings 6 Years Later

Figure 1a shows that acute-phase plasma IL-6 levels were higher in patients who had urinary excretion of α$_1$-microglobulin 6 years later compared with patients showing no excretion of α$_1$-microglobulin. Acute-phase plasma TNF-α levels did not differ between the groups (fig. 1b). Neither acute-phase plasma IL-6 nor TNF-α level had any relation to hypertension, GFR, albuminuria or 24-hour urinary protein excretion 6 years after acute disease (data not shown). Overnight urinary excretion of IL-6 and TNF-α during the acute phase of NE had no association with renal function or urinary protein excretion later (data not shown).

According to our inflammatory score (see Methods), 8 out of 9 patients with elevated urinary excretion of α$_1$-microglobulin belonged to group 2 compared with 1 patient belonging to group 1 ($p = 0.048$). The degree of inflammation had no relation to GFR, hypertension or 24-hour urinary protein excretion 6 years later (data not shown).

Discussion

This is to our knowledge the first report evaluating the influence of clinical severity of acute hantavirus infection on the long-term outcome. In this prospectively gathered group of consecutive patients, we established that the degree of renal insufficiency or any other clinical finding during acute NE had no significant association with renal function, urinary protein excretion or blood pressure 6 years later.

Our assumption was that the severity of acute NE might have an influence on the long-term outcome, since in a previous study we found out that 5 out of 9 patients with hypertension 5 years after acute NE had suffered from severe renal insufficiency during the acute phase [7]. In the present study, this hypothesis was not confirmed, and we actually found that long-term consequences might occur even after a relatively mild course of NE. This notwithstanding, there was a trend toward more severe acute renal failure in those patients who were hypertensive 6 years later, the difference between the groups being however not statistically significant. Here the small sample size may have influenced results.

We have previously found that patients have higher GFR and blood pressure and greater urinary protein excretion 5–6 years after acute interstitial nephritis (AIN) caused by the Puumala hantavirus compared with seronegative healthy controls [7, 8]. The literature offers only limited data on prognostic factors in the long-term outcome of AIN. Most published data concern patients with drug-related AIN. In a review of 3 series of AIN, 71% of cases were drug-related and showed poorer prognosis

with increasing age, but not with peak creatinine concentration [26]. In the present study, the degree of renal insufficiency or age did not affect the long-term outcome.

The clinical course of acute NE is highly variable [2, 3, 5]. The HLA-B8-DR3 haplotype and the carriage of TNF(–308) allele A have been shown to be associated with severe acute NE, although the relationship of TNF-(–308) allele A is probably due to the strong linkage disequilibrium with HLA-B8-DR3 [23, 24]. Furthermore, in a recent Belgian study, high serum creatinine and lower blood platelet count were found to be associated with TNF-α(–238) G/A polymorphism and the lowest ex vivo TNF-α production in patients with acute NE [25]. Among the present limited number of patients, the HLA-B8-DR3 haplotype, the carriage of TNF(–308) allele A or IL-6-(–174) allele G showed no association with the long-term outcome.

Plasma IL-6 concentrations and the degree of inflammation during the acute phase of NE were higher in patients evincing increased urinary excretion of α_1-microglobulin 6 years later compared with those with no urinary excretion of α_1-microglobulin. α_1-Microglobulin is a low-molecular weight protein produced by the liver, freely filtered by glomeruli and reabsorbed by proximal tubuli. It is considered to have immunosuppressive properties in inhibiting the adhesion of neutrophils and monocytes to the vascular endothelium [27]. Increased urinary excretion of α_1-microglobulin can also be a sign of disturbed tubular function [28]. Furthermore, in acute tubular necrosis it may constitute an early predictor of an unfavourable clinical outcome [29], and in membranous nephropathy of the subsequent development of renal insufficiency [30]. In the present study, α_1-microglobulin

excretion during acute NE did not predict urinary protein excretion, renal function or hypertension 6 years later. It has not so far been documented whether the elevated plasma IL-6 concentrations during the acute phase of NE contribute to the tubular function and excretion of α_1-microglobulin several years later. There is controversial evidence from a rat model that the synthesis of α_1-microglobulin may be stimulated by IL-6 [31]. Recently, Vyssoulis et al. [32] have found that urinary α_1-microglobulin is independently associated with circulating acute-phase proteins in patients with newly diagnosed hypertension.

In conclusion, neither the clinical severity of acute NE nor certain host genetic factors had an effect on the 24-hour urinary protein excretion, GFR or blood pressure 6 years later. We were thus unable to identify any specific group of patients who should be strictly followed up after acute NE. Plasma IL-6 concentration and the degree of inflammation during the acute phase of NE were associated with increased urinary α_1-microglobulin excretion after 6 years. The clinical significance of this finding remains to be elucidated.

Acknowledgements

References

1 Brummer-Korvenkontio M, Vapalahti O, Henttonen H, Koskela P, Kuusisto P, Vaheri A: Epidemiological study of nephropathia epidemica in Finland 1989–1996. Scand J Infect Dis 1999;31:427–435.

2 Settergren B: Clinical aspects of nephropathia epidemica (Puumala virus infection) in Europe: a review. Scand J Infect Dis 2000; 32:125–132.

3 Mustonen J, Brummer-Korvenkontio M, Hedman K, Pasternack A, Pietilä K, Vaheri A: Nephropathia epidemica in Finland: a retrospective study of 126 cases. Scand J Infect Dis 1994;26:7–13.

4 Mustonen J, Helin H, Pietilä K, Brummer-Korvenkontio M, Hedman K, Vaheri A, Pasternack A: Renal biopsy findings and clinicopathologic correlations in nephropathia epidemica. Clin Nephrol 1994;41:121–126.

5 Lähdevirta J: Nephropathia epidemica in Finland. A clinical histological and epidemiological study. Ann Clin Res 1971;3:1–54.

6 Niklasson B, Hellsten G, LeDuc J: Hemorrhagic fever with renal syndrome: a study of sequelae following nephropathia epidemica. Arch Virol 1994;137:241–247.

7 Mäkelä S, Ala-Houhala I, Mustonen J, Koivisto AM, Kouri T, Turjanmaa V, Vapalahti O, Vaheri A, Pasternack A: Renal function and blood pressure five years after Puumala virus-induced nephropathy. Kidney Int 2000;58:1711–1718.

8 Miettinen M, Mäkelä S, Ala-Houhala I, Huhtala H, Kööbi T, Vaheri A, Pasternack A, Pörsti I, Mustonen J: Tubular proteinuria and glomerular filtration six years after Puumala hantavirus-induced acute interstitial nephritis. Nephron Clin Pract 2009; 112:c115–c120.

9 Muranyi W, Bahr U, Zeier M, van der Woude FJ: Hantavirus infection. J Am Soc Nephrol 2005;16:3669–3679.

10 Linderholm M, Ahlm C, Settergren B, Waage A, Tärnvik A: Elevated plasma levels of tumour necrosis factor (TNF)-alpha, soluble TNF receptors, interleukin (IL)-6, and IL-10 in patients with hemorrhagic fever with renal syndrome. J Infect Dis 1996;173:38–43.

11 Mäkelä S, Mustonen J, Ala-Houhala I, Hurme M, Koivisto AM, Vaheri A, Pasternack A: Urinary excretion of interleukin-6 correlates with proteinuria in acute Puumala hantavirus-induced nephritis. Am J Kidney Dis 2004;43:809–816.

12 Fishman D, Faulds G, Jeffery R, Mohamed-Ali V, Yudkin JS, Humphries S, Woo P: The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. J Clin Invest 1998;102:1369–1376.

13 Mustonen J, Partanen J, Kanerva M, Pietilä K, Vapalahti O, Pasternack A, Vaheri A: Genetic susceptibility to severe course of nephropathia epidemica caused by Puumala hantavirus. Kidney Int 1996;49:217–221.

14 Plyusnin A, Hörling J, Kanerva M, Mustonen J, Cheng Y, Partanen J, Vapalahti O, Kukkonen SK, Niemimaa J, Henttonen H, Niklasson B, Lundkvist A, Vaheri A: Puumala hantavirus genome in patients with nephropathia epidemica: correlation of PCR positivity with HLA haplotype and link to viral sequences in local rodents. J Clin Microbiol 1997;35:1090–1096.

15 Mustonen J, Partanen J, Kanerva M, Pietilä K, Vapalahti O, Pasternack A, Vaheri A: Association of HLA B27 with benign clinical course of nephropathia epidemica caused by Puumala hantavirus. Scand J Immunol 1998;47:277–279.

16 Vapalahti O, Lundkvist A, Kallio-Kokko H, Paukku K, Julkunen I, Lankinen H, Vaheri A: Antigenic properties and diagnostic potential of Puumala virus nucleocapsid protein expressed in insect cells. J Clin Microbiol 1996;34:119–125.

17 Westman P, Kuismin T, Partanen J, Koskimies S: An HLA-DR typing protocol using group-specific PCR-amplification followed by restriction enzyme digests. Eur J Immunogenet 1993;20:103–109.

18 Wilson AG, di Giovine FS, Blakemore AI, Duff GW: Single base polymorphism in the human tumour necrosis factor alpha (TNF alpha) gene detectable by NcoI restriction of PCR product. Hum Mol Genet 1992;1:353.

19 Hulkkonen J, Pertovaara M, Antonen J, Pasternack A, Hurme M: Elevated interleukin-6 plasma levels are regulated by the promoter region polymorphism of the IL6 gene in primary Sjogren's syndrome and correlate with the clinical manifestations of the disease. Rheumatology (Oxford) 2001;40:656–661.

20 Vervoort G, Veldman B, Berden JH, Smits P, Wetzels JF: Glomerular hyperfiltration in type 1 diabetes mellitus results from primary changes in proximal tubular sodium handling without changes in volume expansion. Eur J Clin Invest 2005;35:330–336.

21 Staessen JA, O'Brien ET, Amery AK, Atkins N, Baumgart P, De Cort P, Degaute JP, Dolenc P, De Gaudemaris R, Enstrom I, et al: Ambulatory blood pressure in normotensive and hypertensive subjects: results from an international database. J Hypertens Suppl 1994;12:S1–S12.

22 Whitworth JA: 2003 World Health Organization (WHO)/International Society of Hypertension (ISH) statement on management of hypertension. J Hypertens 2003;21:1983–1992.

23 Kanerva M, Vaheri A, Mustonen J, Partanen J: High-producer allele of tumour necrosis factor-alpha is part of the susceptibility MHC haplotype in severe Puumala virus-induced nephropathia epidemica. Scand J Infect Dis 1998;30:532–534.

24 Mäkelä S, Mustonen J, Ala-Houhala I, Hurme M, Partanen J, Vapalahti O, Vaheri A, Pasternack A: Human leukocyte antigen-B8-DR3 is a more important risk factor for severe Puumala hantavirus infection than the tumour necrosis factor-alpha(-308) G/A polymorphism. J Infect Dis 2002;186:843–846.

25 Maes P, Clement J, Groeneveld PH, Colson P, Huizinga TW, Van Ranst M: Tumour necrosis factor-alpha genetic predisposing factors can influence clinical severity in nephropathia epidemica. Viral Immunol 2006;19:558–564.

26 Baker RJ, Pusey CD: The changing profile of acute tubulointerstitial nephritis. Nephrol Dial Transplant 2004;19:8–11.

27 Logdberg L, Wester L: Immunocalins: a lipocalin subfamily that modulates immune and inflammatory responses. Biochim Biophys Acta 2000;1482:284–297.

28 Bakoush O, Grubb A, Rippe B, Tencer J: Urine excretion of protein HC in proteinuric glomerular diseases correlates to urine IgG but not to albuminuria. Kidney Int 2001;60:1904–1909.

29 Herget-Rosenthal S, Poppen D, Husing J, Marggraf G, Pietruck F, Jakob HG, Philipp T, Kribben A: Prognostic value of tubular proteinuria and enzymuria in nonoliguric acute tubular necrosis. Clin Chem 2004;50:552–558.

30 Branten AJ, du Buf-Vereijken PW, Klasen IS, Bosch FH, Feith GW, Hollander DA, Wetzels JF: Urinary excretion of beta2-microglobulin and IgG predict prognosis in idiopathic membranous nephropathy: a validation study. J Am Soc Nephrol 2005;16:169–174.

31 Pierzchalski P, Rokita H, Koj A, Fries E, Akerstrom B: Synthesis of alpha 1-microglobulin in cultured rat hepatocytes is stimulated by interleukin-6, leukemia inhibitory factor, dexamethasone and retinoic acid. FEBS Lett 1992;298:165–168.

32 Vyssoulis GP, Tousoulis D, Antoniades C, Dimitrakopoulos S, Zervoudaki A, Stefanadis C: Alpha-1 microglobulin as a new inflammatory marker in newly diagnosed hypertensive patients. Am J Hypertens 2007;20:1016–1021.

Hormonal deficiencies during and after Puumala hantavirus infection

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Abstract Previous reports have described panhypopituitarism associated with severe cases of hemorrhagic fever with renal syndrome (HFRS), but the prevalence of hormonal deficiencies after nephropathia epidemica (NE), a milder form of HFRS, has not been studied. This study was conducted in order to determine the prevalence of hormonal defects in patients with acute NE and during long-term follow-up. Fifty-four patients with serologically confirmed acute NE were examined by serum hormonal measurements during the acute NE, after 3 months, and after 1 to 10 (median 5) years. Thirty out of 54 (56%) patients had abnormalities of the gonadal and/or thyroid axis during the acute NE. After a median follow-up of 5 years, 9 (17%) patients were diagnosed with a chronic, overt hormonal deficit: hypopituitarism was found in five patients and primary hypothyroidism in five patients. In addition, chronic subclinical testicular failure was found in five men. High creatinine levels and inflammatory markers during NE were associated with the acute central hormone deficiencies, but not with the chronic deficiencies. Hormonal defects are common during acute NE and, surprisingly, many patients develop chronic hormonal deficiencies after NE. The occurrence of long-term hormonal defects cannot be predicted by the severity of acute NE.

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Introduction

Nephropathia epidemica (NE) is a form of hemorrhagic fever with renal syndrome (HFRS), caused by Puumala hantavirus (PUUV) [1]. PUUV along with its host, the bank vole (*Myodes glareolus*), is found all over Europe, excluding the Mediterranean region. In Finland, 1,000–3,000 serologically verified cases occur annually and the average PUUV seroprevalence in the population is 5%. Other hantaviruses causing HFRS include Hantaan (HTNV), Dobrava (DOBV), Saaremaa, and Seoul viruses, while several others found in the Americas cause hantavirus cardio-pulmonary syndrome [1]. NE is clinically characterized by fever, headache, nausea, backache, and abdominal pain, as well as transient renal insufficiency [2–4]. Hemorrhages are rare. Complete recovery from NE is the usual outcome, but a convalescent phase with fatigue may last for months [2–4]. Genetic susceptibility seems to determine the severity of NE, as individuals with the HLA B8 DRB1*0301 haplotype have been shown to suffer from a severe form of the disease [5], while the HLA B27 allele correlates with a mild clinical course [6].

There are case reports of hypophyseal hemorrhage and panhypopituitarism in patients with acute NE [7–11].

Postmortem studies of patients with HTNV-induced HFRS, as well as in the few fatal cases of NE, have revealed hemorrhage and necrosis of the pituitary gland in 50–100% of the autopsies [11–13]. The PUUV antigen has been detected in hypophyseal neuroendocrine and endothelial cells of a patient with fatal NE [8]. In a recent retrospective Serbian study of 60 adults with a previous HFRS, a high prevalence of hypopituitarism was identified after recovery from HFRS [14]. Immunological factors including tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) have been suggested to be involved in the pathogenesis of NE [15]. Cytokines produced in inflammatory foci may also cause changes in the endocrine system, including activation of the hypothalamic-pituitary-adrenal (HPA) axis [16, 17].

In the present study, we examined the prevalence of hormonal abnormalities in hospital-treated patients with NE, during both the acute illness and long-term follow-up. We also studied whether the hormonal abnormalities associated with NE were related to the clinical severity of the acute illness, the intensity of the cytokine response (plasma IL-6 and TNF-α levels), or the immunogenotype of the patient (HLA-B8, -DR3, and -B27 alleles).

Materials and methods

Subjects

The original patient group consisted of 70 prospectively collected consecutive patients, hospitalized due to serologically verified acute PUUV infection at Tampere University Hospital, Finland, during the years 1997 to 1999. The specific serological diagnosis was based on an immunoglobulin M-capture enzyme immunoassay and PUUV Sotkamo strain full-length N protein expressed by using the baculovirus system in Sf9 insect cells [18]. In comparison with various other protocols, the assay showed optimal sensitivity and specificity [19]. Fifty-four of the patients attended the 12-month follow-up visit, and they comprised the present study population. The median patient age was 42 years (range 15–70 years), and 37 (69%) of them were male. Fourteen patients had previous diseases: hypertension in four patients, hypothyroidism in two, as well as ankylosing spondyloarthritis, bronchial asthma, aortic valve disease, coeliac disease, sequel of renal tuberculosis, operated cancer of the vocal cords, operated meningioma, and osteoporosis in one each. All of the patients continued their regular medications during the study, including two patients with L-thyroxine substitution for hypothyroidism. None of the patients received corticosteroid therapy, or any other drug that could possibly affect plasma hormone concentrations, except for the two patients on L-thyroxine substitution therapy. One female patient was breast-feeding during acute NE and her estradiol (E2), follicle-stimulating hormone (FSH),

and prolactin (PRL) values were excluded from the analyses. The Ethics Committee of Tampere University Hospital approved the study and every patient gave a written informed consent to participate.

Study protocol

All of the patients were examined during their hospital stay. Detailed medical histories were obtained, and a careful physical examination was performed. All blood specimens were obtained between 0730–0930 h in the morning. Blood samples for plasma creatinine, C-reactive protein (CRP), IL-6, TNF-α, and blood cell counts, as well as 24-h urinary protein excretion were collected on three consecutive mornings after hospital admission. The first blood sample for hormonal analyses was obtained on the first morning of hospital care, and the second sample was taken on the third morning. The highest or the lowest value of each variable measured during hospitalization was designated as the maximum or the minimum value, respectively. All of the serum samples for hormone analyses were stored at −70°C until their use.

The patients were scheduled to an outpatient examination 3 and 12 months after the acute disease. Forty-nine patients attended the 3-month visit (74 to 146 days, median 102 days after admission), and 54 the 12-month visit. The last prearranged follow-up visit was organized in 2004 (4 to 7 years, median 5 years after NE), and 38 patients participated. All serum hormonal levels were analyzed in 2007. Due to the hormonal results, we invited six patients to an additional visit in 2008 (9 to 10 years, median 10 years after NE), and they underwent physical, laboratory, and imaging studies, as appropriate (see the next section).

Methods

Plasma creatinine and CRP concentration, blood cell counts, and 24-h urinary protein excretion were determined by standard laboratory methods. Plasma IL-6 and TNF-α concentrations were determined using enzyme-linked immunosorbent assays (ELISA; PeliKine Compact™ human IL-6 and TNF-α kits, Red Cross Blood Transfusion Service, Amsterdam, The Netherlands). The analyses of the alleles HLA-B8, -DR3, and -B27 were performed as described elsewhere [20].

Serum free thyroxine (fT4) and thyrotropin (TSH) were measured by a chemiluminescent microparticle immunoassay (Abbott Architect i2000 system, Abbott Laboratories, IL, USA), cortisol, PRL, luteinizing hormone (LH), and FSH by fluoroimmunoassay (1235 AutoDELFIA, Wallac Ltd., Turku, Finland), and E2 as well as testosterone (Testo) by radioimmunoassay (1277 GammaMaster, Wallac Ltd.). The six patients invited to an additional visit in 2008 were

also studied for serum levels of growth hormone (GH) by time-resolved fluoroimmunoassay (AutoDELFIA hGH, Wallac Ltd.), insulin-like growth factor 1 (IGF-1) by immunoluminometric assay (DiaSorin Liaison IGF1, Saluggia, Italy), antibodies against thyroid peroxidase (TPOAb) by immunoluminometric assay (Abbott Architect Anti-TPO, Wiesbaden, Germany), as well as for adrenocortical function by the standard 250-μg ACTH stimulation test.

The reference ranges were 9.0–19.0 pmol/l for fT4, 0.4–4.0 mU/l for TSH, 180–680 nmol/l for cortisol, <450 mU/l for PRL in male patients, <600 mU/l in premenopausal women, and <280 mU/l in postmenopausal women. The male reference ranges were 10.4–34.6 nmol/l for Testo and 0.7–6.7 U/l for LH. As the phase of menstrual cycle was not known, E2 below 0.1 nmol/l was regarded as a hypogonadal value for a premenopausal woman. GH reference range was 0–11.5 mU/l, and the age-specific reference values for IGF-1 were 19–65 nmol/l at 18–20 years, 15–45 nmol/l at 21–30 years, 14–36 nmol/l at 31–50 years, 10–29 nmol/l at 51–70 years, and 8–23 nmol/l over 70 years of age. The TPOAb reference range was <6 U/ml. Cortisol response exceeding 550 nmol/l was considered to be normal in the ACTH test.

Peri- and postmenopausal women were not analyzed for defects of the gonadal axis, as they were hypogonadal by definition. However, their FSH and E2 levels were included in the analysis of hormonal alterations between the acute illness and 3 months afterwards. Chronic, overt hypogonadism was defined as Testo (in men) or E2 (in premenopausal women) repeatedly below the reference range one year or more after the acute NE. Chronic, subclinical/compensated testicular failure was diagnosed in a male patient with chronically elevated LH but Testo within the reference range. Chronic, overt hypothyroidism was defined as fT4 repeatedly below 9.0 pmol/l and/or TSH repeatedly above 4.0 mU/l in a patient with chronic thyroiditis, goiter, and/or symptoms suggestive of hypothyroidism [21].

Thyroid ultrasound imaging (Vivid I, GEMS Ultrasound, Tirat Carmel, Israel) was performed by the same investigator (P.J.) on the six patients attending the additional visit in 2008. The hypothalamic-pituitary region was studied by magnetic resonance imaging (MRI; MAGNETOM Trio 3-Tesla scanner, Siemens, Erlangen, Germany) in the patients showing distinct hormonal deficits of central origin.

Statistics

To describe the data, medians (ranges) were given for continuous variables and percentages for categorical variables. Comparisons between the groups were made with the Mann–Whitney U-test for continuous variables, while Pearson's Chi-square test or Fisher's exact test was used for categorical data. Wilcoxon's signed-rank test was used to evaluate changes in serum hormone levels between the acute phase of NE and 3 months afterwards, and between 3 months and 1 year or 5 years, respectively. Correlations were calculated by means of Spearman's rank correlation coefficient. A p-value of less than 0.05 in two-sided testing was considered to be statistically significant. Statistical analyses were performed using SPSS for Windows version 7.5 (SPSS Inc., Chicago, IL, USA).

Results

Clinical and laboratory findings during acute NE

The clinical picture was typical of acute NE in all patients. All had fever lasting for a median of 5 days (range 2 to 14 days). Ten (19%) received empirical antibiotics, but in none of them could a bacterial infection be confirmed. Acute impairment of renal function (maximum plasma creatinine concentration measured during hospital stay >100 μmol/l) was observed in 78% of the patients, and three needed transient dialysis therapy. Thrombocytopenia (blood platelet count $<150 \times 10^9$/l) was present in all patients, but none had major bleeding complications. Hypotension (systolic blood pressure <90 mmHg) was observed on admission in three patients and was corrected by fluid infusion.

Serum hormone levels during the acute phase of NE

Table 1 shows that the serum median cortisol and PRL levels were higher and Testo concentrations lower during the acute phase of NE compared to the corresponding values after 3 months. The serum median LH level did not change. There were, however, remarkable individual differences in the direction and magnitude of serum Testo and LH alterations (Fig. 1). The serum TSH level was slightly higher and FSH concentration lower during the acute phase than after 3 months (Table 1). There were no differences between the median hormone levels (cortisol, PRL, fT4, TSH, Testo, LH, E2, and FSH) measured at 3 months compared to the levels measured after 1 or 5 years (data not shown).

The serum cortisol concentration during acute NE correlated positively with the maximum plasma creatinine and IL-6 levels ($r=0.352$, $p=0.009$, and $r=0.319$, $p=0.019$, respectively), with blood leukocyte count ($r=0.370$, $p=0.006$), and with serum PRL level ($r=0.402$, $p=0.003$). Similarly, the serum PRL level correlated with creatinine and IL-6 levels ($r=0.511$, $p<0.001$, and $r=0.338$, $p=0.012$, respectively), and with blood leukocyte count ($r=0.421$, $p=0.002$).

Eighteen out of 37 (49%) men presented with a low Testo level during the acute phase of NE. Fifteen of them

Table 1 Serum hormone levels during acute nephropathia epidemica (NE) and after 3 months

	Acute phase[a]		At 3 months		p-value
	Median	Range	Median	Range	
All patients (n=54)					
Cortisol (nmol/l)	520	267–1,157	361	163–606	<0.001[c]
Prolactin (mU/l)[b]	266	32–1,364	131	46–550	<0.001[b]
Thyrotropin (mU/l)	1.9	0.1–9.0	1.7	0.02–7.6	0.011[c]
Free thyroxine (pmol/l)	12.2	7.5–18.1	12.8	10.1–17.4	0.526
Males (n=37)					
Testosterone (nmol/l)	9.9	1.1–27.0	17.3	7.8–25.4	<0.001[c]
Luteinizing hormone (U/l)	4.0	0.9–18.0	3.6	2.0–7.9	0.399
Females (n=16)[b]:					
Follicle-stimulating hormone (U/l)	4.7	0.6–40.4	23.7	3.0–73.2	0.001[c]
Estradiol (nmol/l)	0.07	0.02–0.42	0.11	0.04–0.49	0.263

[a] The highest or the lowest serum concentration of each hormone measured during hospitalization, as appropriate

[b] One patient was breast-feeding during acute NE and her E2, FSH, and PRL values were excluded from the analyses (see Subjects and Methods sections)

[c] Statistically significant difference between hormone levels measured in the acute phase and 3 months later (Wilcoxon signed-rank test)

had a coincident serum LH level within the reference range, and three had an increased LH level. In addition, 5 (14%) men had an elevated LH concentration together with a Testo level within the reference range. None of the eight male patients with acute primary hypogonadism (elevated LH) complained of testicular pain. The minimum Testo concentration correlated inversely with age and leukocytosis ($r=-0.341$, $p=0.039$, and $r=-0.493$, $p=0.002$, respectively), and with maximum creatinine, IL-6, CRP, and PRL

levels ($r=-0.437$, $p=0.007$; $r=-0.411$, $p=0.011$; $r=-0.352$, $p=0.032$, $r=-0.363$, $p=0.027$, respectively), but not with body mass index (BMI) ($r=-0.009$, $p=0.957$). Three out of six premenopausal women (aged 16, 21, and 37 years) presented with low E2 and FSH levels during the acute phase of NE (E2 range 0.04–0.05 nmol/l, and FSH range 0.08–0.8 U/l).

Two patients had a low serum fT4 level together with a low-normal TSH level during the acute phase. Five out of
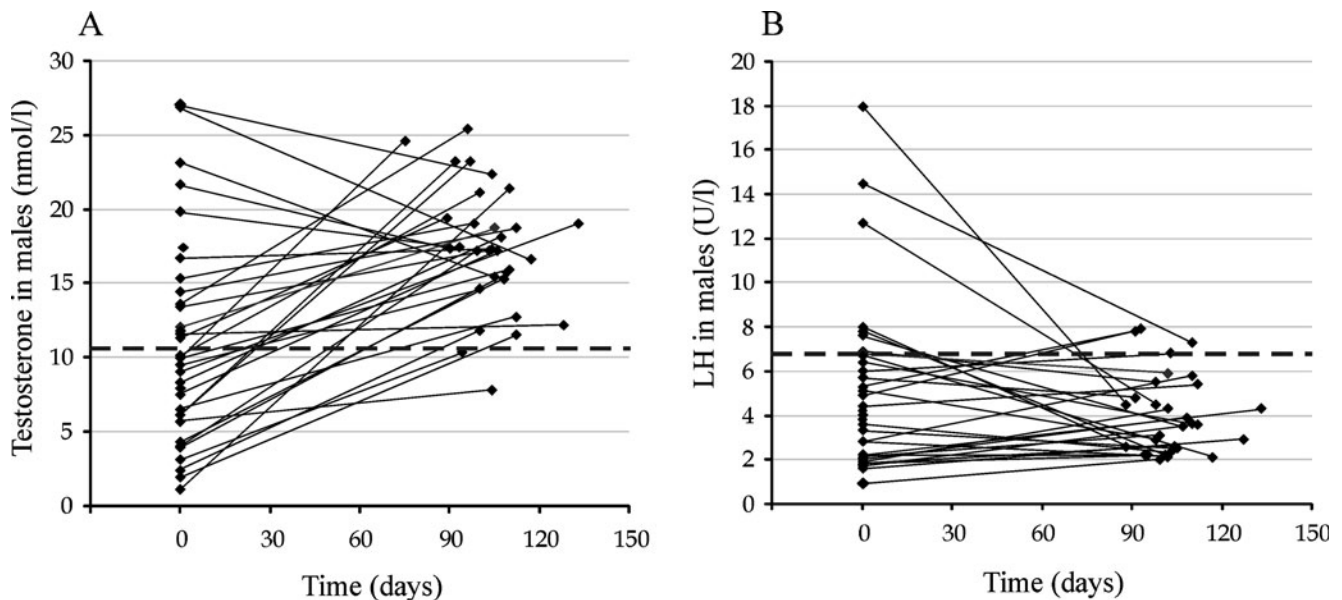
Fig. 1 Serum testosterone (**A**) and luteinizing hormone (LH) (**B**) levels measured in 37 men during acute nephropathia epidemica (NE) and after 3 months. Testosterone levels were below the reference range in half of the men during acute NE, while LH levels were within the

reference range in the majority, indicating that the hypogonadism was mostly of central origin. The reference ranges were 10.4–34.6 nmol/l for testosterone (*vertical line* showing lower reference limit) and 0.7–6.7 U/l for LH (*vertical line* showing higher reference limit)

54 (9%) patients presented with an increased serum TSH level together with fT4 within the low-normal range; one of them had a previously diagnosed primary hypothyroidism, and she used L-thyroxine substitution throughout the study. Serum fT4 and TSH levels measured during acute NE did not correlate with age, BMI, plasma creatinine, CRP, or cytokine levels (data not shown).

Altogether, 30 out of 54 (56%) patients had abnormalities of the gonadal and/or thyroid axis during the acute phase of NE. Central hormonal defects were found in 19 patients, while 13 patients had a primary deficit. As shown in Table 2, the patients with central hormonal deficiencies had a higher plasma creatinine concentration and blood leukocyte count than the patients with normal hormone levels.

There were no differences in the clinical picture or basic laboratory findings during acute NE between the patients with primary hormonal deficiencies and those with normal hormone levels (data not shown), and neither did the cytokine levels or HLA-B8, -DR3, and -B27 alleles predict the acute primary hormone deficits.

Hormonal deficiencies one to ten years after NE

During a median follow-up of 5 years, 9 out of 54 (17%) NE patients presented with chronic, overt hormonal deficits. Table 3 shows the serial plasma hormone levels of these patients. The clinical diagnoses of overt hormonal deficiencies were based both on the hormone measurements and on the clinical signs and symptoms of the patients.

One of the two patients with hormone levels suggestive of combined pituitary hormone defects, a 43-year-old male

patient (patient 1, Table 3), presented with low testosterone levels and declining fT4 levels within the low reference range during acute NE, as well as after 3 and 12 months (Testo 7.1–7.8–7.6 nmol/l, LH 2.8–2.1–1.8 U/l, fT4 11.9–10.6–10.4 pmol/l, and TSH 2.8–3.8–1.9 mU/l, respectively). He died for an unknown reason soon after his 1-year follow-up visit. The hormone levels, indicating central hypogonadism and possible hypothyroidism, were analyzed only after his death. The overt hormonal deficiencies of the other patients had also gone unnoticed until the patients were invited to the additional visit in 2008. Only one female patient had been started on L-thyroxine, due to overt hypothyroidism diagnosed 9 years after the acute NE (patient 8, Table 3).

There were no differences in the age or gender distribution, clinical picture, or basic laboratory findings during acute NE between those nine patients who presented with chronic hormone deficiencies after NE and those with normal hormone levels (Table 4). None of the three patients who were hypotensive on admission developed chronic hormonal deficiencies. The prevalence of HLA-B8, -DR3, and -B27 alleles did not differ between the groups (data not shown).

Imaging studies during the additional visit in 2008

Thyroid ultrasound examination showed diffuse or patchy hypoechogenicity, pertinent with thyroiditis, in the five patients with primary hypothyroidism, while TPOAb were measurable in only two of them. A sellar MRI scan was performed during the additional visit in 2008 in two men whose symptoms and hormone levels suggested central

Table 2 Clinical and laboratory findings of the 19 patients diagnosed with central hormonal defects during the acute phase of NE compared to those of the 23 patients with normal hormone levels

Finding	Central hormonal defects ($n=19$)		Normal hormone levels ($n=23$)		p-value
	Median	Range	Median	Range	
Age (years)	41	17–62	41	24–70	0.909
Gender (M/F)	15/4		13/10		0.125
Body mass index (kg/m^2)	25	19–37	26	19–42	0.456
Creatinine max (μmol/l)	368	79–749	208	76–878	0.029[a]
Leukocytes max (10^9/l)	13.4	4.4–22.8	9.2	6.7–23.4	0.033[a]
Platelets min (10^9/l)	62	13–127	53	18–136	0.869
C-reactive protein max (mg/l)	78	22–213	67	27–136	0.791
Plasma IL-6 max (pg/ml)	19.6	5.9–40.7	13.5	3.3–59.1	0.052
Plasma TNF-α max (pg/ml)	3.4	1.4–26.9	3.8	1.4–37.4	0.693
Proteinuria max (g/day)	1.8	0.2–9.5	2.0	0.3–17.8	0.990
Duration of hospital stay (days)	8	5–13	7	3–11	0.134

M = male; F = female; IL = interleukin; TNF-α = tumor necrosis factor alpha; min = minimum; max = maximum

[a] Statistically significant difference between patients with acute central hormone deficits and those with normal hormone levels. The Mann–Whitney U-test was used for continuous variables and Pearson's Chi-square test was used for gender

Table 3 Serial plasma hormone levels of nine NE patients who presented with chronic, overt hormonal deficits during a median follow-up of 5 years. The hormone levels presented in the table were measured during the acute phase of NE (first value in each series) and on the follow-up visits 1 to 10 years later (second and third value of each series). Abnormal values are printed in *bold*

Patient	Gender/age during acute NE (years)	Duration of follow-up (years)	Testosterone in males and E2 in females (nmol/l)	LH in males and FSH in females (U/l)	fT4 (pmol/l)	TSH (mU/l)	Clinical diagnosis
1	M/43	1[a]	**7.1–7.6**	2.8–1.8	11.9–10.4	2.8–1.9	CHG
2	M/41	9	11.6–**8.1–7.4**	1.6–2.0–2.6	12.5–10.4–10.4	1.2–1.0–0.9	CHG
3	M/59	5	**1.9**–11.5–**7.6**	1.7–3.6–2.3	11.0–11.4–12.1	1.3–1.7–1.5	CHG
4	M/52	1[a]	**7.9**–9.8	2.5–2.8	14.5–13.3	2.3–1.5	CHG
5	M/42	9	11.3–15.7–21.9	**18.0**–5.5–**6.8**	11.2–13.9–13.4	1.9–**4.3**–**5.0**	PHT[b]
6	M/58	9	12.5–15.3–10.7	**6.9**–6.7–**9.2**	14.0–12.8–12.0	3.7–**4.3**–**5.7**	PHT
7	M/60	7	**1.1**–19.2–21.1	2.8–5.0–4.0	9.4–10.9–11.7	1.7–**5.8**–**9.9**	PHT
8	F/17	9	**0.05**–**0.07**–**<0.06**	0.6–2.8–0.4	16.2–11.5–12.5	3.5–2.7–**6.6**[c]	CHG, PHT
9	F/47	9	0.05–**<0.02**–**<0.06**	2.6–41.5–39.8	9.5–9.9–9.4	**9.0**–11.5–**16.1**	PHT

CHG = central hypogonadism; PHT = primary hypothyroidism; E2 = estradiol; LH = luteinizing hormone; FSH = follicle-stimulating hormone; fT4 = free thyroxine; TSH = thyrotropin

[a] Only the hormone levels measured during acute NE and 1 year later are shown for the two patients whose follow-up ended at the 1-year visit

[b] And a possible growth hormone (GH) deficiency: an undetectable GH level and insulin-like growth factor-1 6.5 nmol/l (reference range 10–29 nmol/l)

[c] On L-thyroxine treatment

The reference ranges were 10.4–34.6 nmol/l for testosterone and 0.7–6.7 U/l for LH in men. An E2 level below 0.1 nmol/l was considered to be hypogonadal in premenopausal women, as the phase of menstrual cycle was not known. Peri- and postmenopausal women were not analyzed for gonadal defects, as they were hypogonadal by definition. The reference range for fT4 was 9.0–19.0 pmol/l and for TSH, it was 0.4–4.0 mU/l

hormonal defects. One had an apparent GH deficiency (patient 5, Table 3), and the other was a 41-year-old patient who developed a marked secondary hypogonadism and a probable secondary hypothyroidism (patient 2, Table 3). The MRI scans showed no pathology of the hypothalamic-pituitary region in either of them.

Discussion

To our knowledge, this is the first study on hormonal alterations occurring during and after NE. Serum cortisol and PRL levels were higher and serum Testo concentrations lower during acute NE compared to the corresponding

Table 4 Clinical and laboratory findings during the acute phase of NE in the nine patients who developed chronic hormonal defects after NE compared to those in the 45 patients whose hormone levels remained normal during the follow-up

	Chronic hormonal defects (n=9)		No chronic defects (n=45)		p-value
	Median	Range	Median	Range	
Age (years)	47	17–60	39	15–70	0.194
Gender (M/F)	7/2		30/15		0.512
Body mass index (kg/m^2)	26	21–37	25	19–42	0.347
Creatinine max (μmol/l)	380	98–725	213	76–878	0.190
Leukocytes max (10^9/l)	11.2	5.7–20.4	10.3	4.4–23.4	0.926
Platelets min (10^9/l)	54	17–139	62	3–136	0.898
C-reactive protein max (mg/l)	72	25–213	67	20–136	0.423
Plasma IL-6 max (pg/ml)	19.9	5.0–43.0	14.2	3.3–59.1	0.102
Plasma TNF-α max (pg/ml)	2.8	1.4–8.7	3.7	1.4–37.4	0.267
Proteinuria max (g/day)	0.9	0.3–3.9	2.0	0.1–17.8	0.114
Duration of hospital stay (days)	6	5–13	7	3–12	0.883

The Mann–Whitney U-test was used for continuous variables and Pearson's Chi-square test was used for gender

M = male; F = female; IL = interleukin; TNF-α = tumor necrosis factor alpha; min = minimum; max = maximum

values after 3 months. These acute hormonal alterations were related to the severity of acute renal insufficiency and inflammation. After one to ten years, surprisingly, many patients suffered from chronic hormonal deficits of either central or peripheral origin. The chronic defects could not be predicted by renal insufficiency, degree of inflammation, or other markers of the clinical severity of acute NE, or by the host genetic factors studied.

Approximately half of the men and premenopausal women presented with low serum gonadal hormone levels during the acute phase of NE. Serum Testo levels, in general, increased during a 3-month follow-up, while concomitant LH levels remained stable, indicating that the acute hypogonadism was transient and of central origin in most cases. The degree of hypothalamic-pituitary-gonadal axis suppression, as well as the increase in plasma cortisol production, are known to be related to the severity of acute illness [22, 23]. Also, in our patients with acute NE, low Testo levels and high cortisol and PRL levels were related to the degree of inflammation and the severity of acute renal failure. As the kidneys are involved in the elimination of PRL and corticosteroids [24, 25], the acute impairment of renal function during NE may have resulted in diminished clearance of PRL and cortisol, contributing to their increased serum levels.

We also found eight male patients with acute primary hypogonadism during PUUV infection. Three out of these eight acute testicular failures developed into chronic, compensated failures, i.e., Testo levels remained within the normal range, while LH levels were chronically elevated. In addition, two cases of chronic, compensated hypogonadism developed during the follow-up, without testicular dysfunction during the acute NE. There are no previous reports of clinical orchitis or primary hypogonadism in hantaviral diseases. However, the possibility remains that PUUV infection may have contributed to the pathogenesis of acute and chronic primary hypogonadism, which were over-represented [26, 27] in this relatively young group of patients (median age in males 39 years, the youngest patient with chronic hypogonadism 30 years).

Primary hypothyroidism was remarkably frequent in our patients with NE. Two patients had been diagnosed with hypothyroidism prior to NE, and five more diagnoses of primary hypothyroidism were made during the follow-up. The overall prevalence of primary hypothyroidism thus mounted up to 24% in the female patients and 8% in the males, which are about three-fold prevalences compared to those recently reported for similar age groups in the general population [28–30]. All of the patients diagnosed with primary hypothyroidism in the present study showed a hypo-echogenic structure of the thyroid on ultrasound examination, suggesting chronic thyroiditis [31, 32]. TPOAb were present in only two of the five "new" cases of hypothyroidism, which

may indicate that in the other three cases, the thyroiditis was not of autoimmune origin, but was possibly induced by the PUUV infection. The primary thyroid, or gonadal, defects did not relate to age, gender, or the degree of renal insufficiency or inflammation during acute NE. A high level of vigilance for possible signs of thyroid or gonadal dysfunction seems warranted in patients with a history of previous NE.

Seventeen percent of the present study patients were diagnosed with a chronic, overt hormonal deficit. In a recent retrospective Serbian study, the prevalence of any endocrine deficiency 2 years after HFRS was 18% [14]. It is noteworthy that the patients in the Serbian study had a more severe HFRS than our patients. In the present study, only three patients needed dialysis treatment, while 57% of the Serbian patients were dialyzed. PUUV and DOBV coexist in the Balkan area, and induce HFRS with significant differences in severity [33, 34]. DOBV-infected patients have more frequently suffered from acute renal failure requiring dialysis treatment, shock, severe thrombo-cytopenia, and hemorrhagic complications than patients with PUUV [33, 34]. In the present study, chronic hormonal defects could not be predicted by the severity of acute NE. Hence, it is worth noticing that chronic hormonal deficits might occur even after a relatively mild hantaviral infection.

Previous case reports of severe HFRS have described hypophyseal hemorrhage and panhypopituitarism associated with PUUV and HTNV infections [7–11, 35–38]. The development of hypophyseal hemorrhage and subsequent panhypopituitarism has varied from acute to subacute. The mechanisms of pituitary hemorrhage are unknown, although hypovolemic shock and thrombocytopenia may contribute to the pathogenesis. In the present patients, thrombocytopenia or hypotension during acute NE did not correlate with either acute-phase or long-term hormonal abnormalities. Neither were there any MRI findings pertinent with previous hypophyseal or hypothalamic hemorrhage in the patients with central hormonal defects. It is likely that the secondary hormone deficiencies found in the present series of NE patients developed by mechanisms other than an acute hemorrhagic disruption of the pituitary.

Headache, vertigo, visual disturbances, and other signs of central nervous system involvement occur frequently in NE [2, 3, 39, 40], and cases of PUUV-induced meningo-encephalitis have been reported [2, 3, 41, 42]. The presence of PUUV RNA in cerebrospinal fluid in a patient with acute NE has been confirmed [43]. It has recently been shown that infections of the central nervous system may cause transient or permanent hypothalamic and/or pituitary dys-function [44]. Viral meningoencephalitis or hypophysitis may also have contributed to the central hormone defects frequently found in our patients.

An important feature in hantaviral infections is a universally increased capillary permeability, causing tissue

edema and hypotension [1]. Immunological factors, including the overproduction of pro-inflammatory cytokines during acute infection, are probably involved in the pathogenesis and might result in immune-mediated damage to the endothelial cells [15]. The endothelial damage and increased vascular permeability may have been involved in the pathogenesis of central and peripheral hormonal defects of the NE patients. A tight interaction between the immune and endocrine systems, mainly through the HPA axis [16, 17], is also likely to play a central role in the hormonal alterations, at least during acute NE.

Our study has some limitations. First, due to the retrospective nature of the hormonal analyses, we did not have complete data on the possible signs and symptoms related to hormonal alterations during and after NE. This may have resulted in underestimation of the hormonal sequelae of NE, as milder forms of pituitary failure cannot be diagnosed by means of basal hormone levels alone. Stimulatory tests of the GH-IGF-1 axis or the HPA axis could have revealed more subtle hormonal defects, over and above the prevalence of overt hormonal failures reported here. In addition, it would have been valuable to include patients with other viral infections as controls. These limitations, however, should be weighed against the strengths of investigating a prospectively gathered group of consecutive patients both during the acute phase of NE and repeatedly during the long-term follow-up. The findings of the present study give rise to prospective, controlled studies to verify the prevalence of hormonal defects after hantaviral diseases, and to clarify the pathogenesis of these hormonal alterations.

In summary, hormonal alterations were common during acute NE, and the acute hormone deficits of central origin correlated with the clinical severity of NE. Chronic hormonal defects, however, could not be predicted by the severity of acute NE, and chronic deficits emerged even after a relatively mild course of NE. Patients with a history of NE should be readily investigated for hormonal deficiencies, in view of the high prevalence of chronic central and peripheral hormone defects in these patients.

References

1. Vapalahti O, Mustonen J, Lundkvist Å, Henttonen H, Plyusnin A, Vaheri A (2003) Hantavirus infections in Europe. Lancet Infect Dis 3(10):653–661

2. Lähdevirta J (1971) Nephropathia epidemica in Finland. A clinical histological and epidemiological study. Ann Clin Res 3:1–54

3. Mustonen J, Brummer-Korvenkontio M, Hedman K, Pasternack A, Pietilä K, Vaheri A (1994) Nephropathia epidemica in Finland: a retrospective study of 126 cases. Scand J Infect Dis 26(1):7–13

4. Settergren B, Juto P, Trollfors B, Wadell G, Norrby SR (1989) Clinical characteristics of nephropathia epidemica in Sweden: prospective study of 74 cases. Rev Infect Dis 11(6):921–927

5. Mustonen J, Partanen J, Kanerva M, Pietilä K, Vapalahti O, Pasternack A, Vaheri A (1996) Genetic susceptibility to severe course of nephropathia epidemica caused by Puumala hantavirus. Kidney Int 49(1):217–221

6. Mustonen J, Partanen J, Kanerva M, Pietilä K, Vapalahti O, Pasternack A, Vaheri A (1998) Association of HLA B27 with benign clinical course of nephropathia epidemica caused by Puumala hantavirus. Scand J Immunol 47(3):277–279

7. Forslund T, Saltevo J, Anttinen J, Auvinen S, Brummer-Korvenkontio M, Korhonen A, Poutiainen M (1992) Complications of nephropathia epidemica: three cases. J Intern Med 232 (1):87–90

8. Hautala T, Sironen T, Vapalahti O, Pääkkö E, Särkioja T, Salmela PI, Vaheri A, Plyusnin A, Kauma H (2002) Hypophyseal hemorrhage and panhypopituitarism during Puumala virus infection: magnetic resonance imaging and detection of viral antigen in the hypophysis. Clin Infect Dis 35(1):96–101

9. Sane T, Färkkilä M (2002) Hypopituitarism and hepatitis as complications of nephropathia epidemica. Duodecim 118(5):457–461

10. Settergren B, Boman J, Linderholm M, Wiström J, Hägg E, Arvidsson PA (1992) A case of nephropathia epidemica associated with panhypopituitarism and nephrotic syndrome. Nephron 61 (2):234–235

11. Valtonen M, Kauppila M, Kotilainen P, Lähdevirta J, Svartbäck CM, Kosunen O, Nurminen J, Sarkkinen H, Brummer-Korvenkontio M (1995) Four fatal cases of nephropathia epidemica. Scand J Infect Dis 27(5):515–517

12. Hullinghorst RL, Steer A (1953) Pathology of epidemic hemorrhagic fever. Ann Intern Med 38(1):77–101

13. Lukes RJ (1954) The pathology of thirty-nine fatal cases of epidemic hemorrhagic fever. Am J Med 16(5):639–650

14. Stojanovic M, Pekic S, Cvijovic G, Miljic D, Doknic M, Nikolic-Djurovic M, Micic D, Hrvacevic R, Nesic V, Popovic V (2008) High risk of hypopituitarism in patients who recovered from hemorrhagic fever with renal syndrome. J Clin Endocrinol Metab 93(7):2722–2728

15. Kanerva M, Mustonen J, Vaheri A (1998) Pathogenesis of Puumala and other hantavirus infections. Rev Med Virol 8(2):67–86

16. Imura H, Fukata J (1994) Endocrine-paracrine interaction in communication between the immune and endocrine systems. Activation of the hypothalamic-pituitary-adrenal axis in inflammation. Eur J Endocrinol 130(1):32–37

17. Turnbull AV, Rivier CL (1999) Regulation of the hypothalamic-pituitary-adrenal axis by cytokines: actions and mechanisms of action. Physiol Rev 79(1):1–71

18. Vapalahti O, Lundkvist Å, Kallio-Kokko H, Paukku K, Julkunen I, Lankinen H, Vaheri A (1996) Antigenic properties and diagnostic potential of Puumala virus nucleocapsid protein expressed in insect cells. J Clin Microbiol 34(1):119–125

19. Sjölander KB, Elgh F, Kallio-Kokko H, Vapalahti O, Hägglund M, Palmcrantz V, Juto P, Vaheri A, Niklasson B, Lundkvist Å (1997) Evaluation of serological methods for diagnosis of Puumala hantavirus infection (nephropathia epidemica). J Clin Microbiol 35(12):3264–3268

20. Westman P, Kuismin T, Partanen J, Koskimies S (1993) An HLA-DR typing protocol using group-specific PCR-amplification

followed by restriction enzyme digests. Eur J Immunogenet 20 (2):103–109

21. Baskin HJ, Cobin RH, Duick DS, Gharib H, Guttler RB, Kaplan MM, Segal RL; American Association of Clinical Endocrinologists (2002) American Association of Clinical Endocrinologists medical guidelines for clinical practice for the evaluation and treatment of hyperthyroidism and hypothyroidism. Endocr Pract 8(6):457–469

22. Jurney TH, Cockrell JL Jr, Lindberg JS, Lamiell JM, Wade CE (1987) Spectrum of serum cortisol response to ACTH in ICU patients. Correlation with degree of illness and mortality. Chest 92(2):292–295

23. Spratt DI, Cox P, Orav J, Moloney J, Bigos T (1993) Reproductive axis suppression in acute illness is related to disease severity. J Clin Endocrinol Metab 76(6):1548–1554

24. Bauer AG, Wilson JH, Lamberts SW (1980) The kidney is the main site of prolactin elimination in patients with liver disease. J Clin Endocrinol Metab 51(1):70–73

25. Whitworth JA, Stewart PM, Burt D, Atherden SM, Edwards CR (1989) The kidney is the major site of cortisone production in man. Clin Endocrinol (Oxf) 31(3):355–361

26. Gray A, Feldman HA, McKinlay JB, Longcope C (1991) Age, disease, and changing sex hormone levels in middle-aged men: results of the Massachusetts Male Aging Study. J Clin Endocrinol Metab 73(5):1016–1025

27. Härkönen K, Huhtaniemi I, Mäkinen J, Hübler D, Irjala K, Koskenvuo M, Oettel M, Raitakari O, Saad F, Pöllänen P (2003) The polymorphic androgen receptor gene CAG repeat, pituitary-testicular function and andropausal symptoms in ageing men. Int J Androl 26(3):187–194

28. Bjoro T, Holmen J, Krüger O, Midthjell K, Hunstad K, Schreiner T, Sandnes L, Brochmann H (2000) Prevalence of thyroid disease, thyroid dysfunction and thyroid peroxidase antibodies in a large, unselected population. The Health Study of Nord-Trondelag (HUNT). Eur J Endocrinol 143(5):639–647

29. Hollowell JG, Staehling NW, Flanders WD, Hannon WH, Gunter EW, Spencer CA, Braverman LE (2002) Serum TSH, T(4), and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). J Clin Endocrinol Metab 87(2):489–499

30. Vanderpump MPJ (2005) The epidemiology of thyroid diseases. JB Lippincott-Raven, Philadelphia, pp 398–406

31. Hegedüs L (2001) Thyroid ultrasound. Endocrinol Metab Clin North Am 30(2):339–360

32. McGahan JP, Goldberg BB (2008) Diagnostic ultrasound. Informa Healthcare, New York-London

33. Avsic-Zupanc T, Petrovec M, Furlan P, Kaps R, Elgh F, Lundkvist Å (1999) Hemorrhagic fever with renal syndrome in the Dolenjska region of Slovenia—a 10-year survey. Clin Infect Dis 28(4):860–865

34. Markotić A, Nichol ST, Kuzman I, Sanchez AJ, Ksiazek TG, Gagro A, Rabatić S, Zgorelec R, Avsic-Zupanc T, Beus I, Dekaris D (2002) Characteristics of Puumala and Dobrava infections in Croatia. J Med Virol 66(4):542–551

35. Kim NH, Cho JG, Ahn YK, Lee SU, Kim KH, Cho JH, Kim HG, Kim W, Jeong MH, Park JC, Kang JC (2001) A case of torsade de pointes associated with hypopituitarism due to hemorrhagic fever with renal syndrome. J Korean Med Sci 16(3):355–359

36. Park JE, Pyo HJ (1996) Delayed onset of diuresis in a patient with acute renal failure due to hemorrhagic fever with renal syndrome who also developed anterior hypopituitarism. Clin Nephrol 46 (2):141–145

37. Pekic S, Cvijovic G, Stojanovic M, Kendereski A, Micic D, Popovic V (2005) Hypopituitarism as a late complication of hemorrhagic fever. Endocrine 26(2):79–82

38. Suh DC, Park JS, Park SK, Lee HK, Chang KH (1995) Pituitary hemorrhage as a complication of hantaviral disease. AJNR Am J Neuroradiol 16(1):175–178

39. Ahlm C, Lindén C, Linderholm M, Alexeyev OA, Billheden J, Elgh F, Fagerlund M, Zetterlund B, Settergren B (1998) Central nervous system and ophthalmic involvement in nephropathia epidemica (European type of haemorrhagic fever with renal syndrome). J Infect 36(2):149–155

40. Alexeyev OA, Morozov VG (1995) Neurological manifestations of hemorrhagic fever with renal syndrome caused by Puumala virus: review of 811 cases. Clin Infect Dis 20(2):255–258

41. Bergmann F, Krone B, Bleich S, Prange H, Paulus W (2002) Encephalitis due to a hantavirus infection. J Infect 45(1):58–59

42. Launes J, Hautanen A (1988) Nephropathia epidemica encephalitis. Acta Neurol Scand 78(3):234–235

43. Mähönen SM, Sironen T, Vapalahti O, Pääkkö E, Hautala N, Ilonen J, Glumoff V, Vainio O, Kauma H, Vaheri A, Plyusnin A, Hautala T (2007) Puumala virus RNA in cerebrospinal fluid in a patient with uncomplicated nephropathia epidemica. J Clin Virol 40(3):248–251

44. Schaefer S, Boegershausen N, Meyer S, Ivan D, Schepelmann K, Kann PH (2008) Hypothalamic-pituitary insufficiency following infectious diseases of the central nervous system. Eur J Endocrinol 158(1):3–9

Glomerulonephritis emerging shortly after Puumala hantavirus infection: a report on 7 patients

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Abstract. Aims: Nephropathia epidemica (NE) is mild type of hemorrhagic fever caused by Puumala (PUU) hantavirus. Renal biopsy typically shows acute tubulointerstitial nephritis and complete recovery is the usual outcome. We previously described 5 patients with membranoproliferative glomerulonephritis (MPGN) after acute NE. We now report on 7 more patients who developed biopsy-confirmed glomerulonephritis (GN) during the convalescent phase of NE. Material and methods: We present case histories of 7 patients with nephrotic-range proteinuria concomitant with hematuria after serologically verified NE. Results: Renal biopsy specimens disclosed MPGN in 5 patients, membranous GN (MGN) in 1 and mesangial GN (MesGN) in 1. All patients achieved remission of nephrotic syndrome within a median time of 0.6 years (range 0.5 – 5.5 y). The median follow-up time was 1.7 years (0.7 – 15.6 y). Conclusions: As a rare phenomenon, nephrotic syndrome may emerge during the convalescent phase of acute PUU hantavirus infection. In most cases the prognosis of GN caused by NE seems to be favorable.

Introduction

Nephropathia epidemica (NE) is a mild type of hemorrhagic fever with renal syndrome (HFRS). The causative agent is a Puumala (PUU) hantavirus carried by bank voles (*Myodes glareolus*) [1, 2]. The typical, but unspecific symptoms of acute NE are fever, headache, backache and abdominal pain [3, 4, 5]. Oliguria followed by polyuria is a common clinical finding. Acute worsening of renal function together with thrombocytopenia and leukocytosis is usual. Proteinuria and hematuria are detected upon urinalysis. Although proteinuria is often in the nephrotic range, the typical histopathological finding in renal biopsy is acute tubulointerstitial nephritis without specific changes in glomeruli [3, 6]. Extensive interstitial hemorrhage in the outer medulla is characteristic for hantavirus nephropathy [7]. The clinical severity of the disease varies, but complete recovery is the usual outcome [3, 4, 5, 8].

The first published report of a possible association between hantavirus infection and glomerulonephritis (GN) was an account of a patient with diffuse proliferative GN [9]. In a previous renal biopsy material of 86 patients with acute NE there was 1 case with IgA nephropathy (IgAN), but no other cases with GN [6]. Probably both associations are incidental. However, we have previously reported on 5 patients suffering from membranoproliferative GN (MPGN) shortly after serologically verified acute NE [10]. Here we describe 7 more cases of GN diagnosed during the convalescent phase of NE.

Methods

The diagnosis of a recent PUUV infection was confirmed serologically either by detecting immunoglobulin M class antibodies by

Table 1.　Clinical and laboratory data at the time of renal biopsy.

Patient	Age/sex	Blood pressure (mm/Hg)	Urinary protein excretion (g/d)	Serum creatinine (µmol/l)	Serum albumin (g/l)	Time from acute NE to renal biopsy (weeks)	Renal biopsy finding
1	48/F	190/106	14.9	200	22	4	MPGN
2	40/M	131/82	7.3	91	20	5	MPGN
3	39/F	170/100	9.8	62	26	5	MPGN
4	48/F	170/100	27.2	233	17	4	MPGN
5	59/F	175/100	4.3	186	26	5	MPGN
6	28/M	130/80	21.2	90	13	56	MGN
7	54/M	170/90	11.0	108	32	20	MesGN

MPGN = membranoproliferative glomerulonephritis; MGN = membranous glomerulonephritis; GN = glomerulonephritis; MesGN = mesangial glomerulonephritis.

Table 2.　Renal biopsy findings in seven patients with nephrotic syndrome diagnosed shortly after acute Puumala hantavirus infection.

Patient	Light microscopic finding	Glomerular immunofluorescence finding
1	MPGN[a] Type 1 with infiltrating polymorphonuclear leukocytes in glomeruli	NA
2	MPGN[a] Type 1 and slight mononuclear cell infiltration in the interstitium	Capillary and mesangial IgG++, IgM++ and C3++
3	MPGN[a] Type 1	Capillary IgG++, IgM+, C1q++ and C3+++
4	MPGN[a] Type 1 with slight interstitial edema and inflammatory cell infiltration	Capillary IgM+ and C3+++
5	MPGN[a] Type 1 with interstitial edema	Capillary IgG++ and C3+++, mesangial C3+++
6	MGN; spikes and vacuoles on capillary basement membrane without hypercellularity	Capillary IgG+++ and C1q+
7	MesGN; increased mesangial matrix	Mesangial IgG+, IgM+, IgA+ and C1q ++

[a]The morphological diagnosis of MPGN Type 1 was based on increased mesangial cellularity and matrix, accentuated lobulation, and thickening of capillary walls together with double contours of capillary basement membranes. MPGN = membranoproliferative glomerulonephritis; MGN = membranous GN; MesGN = mesangial GN; NA = not available.

immunofluorescence assays [11] or by rapid immunochromatographic test [12].

The renal biopsy specimens were processed for light-microscopic, immunofluorescence (IF) and electronmicroscopic studies using standard techniques.

Remission of nephrotic syndrome was defined as a reduction in proteinuria to ≤ 300 mg/day [13, 14]. The time-point of remission was calculated from the first clinical symptoms and signs of glomerulonephritis.

Results

Case histories

Patient 1 is a 48-year-old woman with uncomplicated Type 1 diabetes treated with glargine and aspart insulins. Two years before acute NE her urinary albumin/creatinine ratio had been normal. During acute NE, her serum creatinine rose to 551 µmol/l and she had oliguria. One week after hospital discharge she

Table 3. Treatment of the glomerulonephritis and follow-up characteristics during the last visit.

Patient	Renal biopsy finding[a]	Treatment[b]	Time to remission/ duration of follow-up (years)	Blood pressure (mm/Hg)	Proteinuria (g/d)	Hematuria	Serum creatinine (µmol/l)
1	MPGN	Di	1.3/1.6	140/90	< 0.30	NA	79
2	MPGN	ACEi	0.5/0.7	130/78	0.24	+	80
3	MPGN	Di	0.6/1.7	130/90	0.17	neg	49
4	MPGN	Di, Wa, Co	0.6/2.1	120/80	0.09	+	75
5	MPGN	Di, Wa, Co	0.5/0.8	150/100	0.15	+	114
6	MGN	ACEi, Di, Co, CP, Wa	5.5/15.6	120/80	0.30	neg	80
7	MesGN	ARB	2.1/3.8	120/90	0.30	neg	86

[a]MPGN = membranoproliferative glomerulonephritis; MGN = membranous glomerulonephritis; GN = glomerulonephritis; MesGN = mesangial glomerulonephritis; [b]Di = diuretic; ACEi = angiotensin converting enzyme inhibitor; Wa = warfarin; Co = corticosteroid; ARB = angiotensin receptor blocker; CP = cyclophosphamide; NA = not available.

began to feel fatigued and developed peripheral edema. Serum creatinine was 200 µmol/l and urinanalysis revealed proteinuria concomitant with microscopic hematuria (Table 1). Renal biopsy showed MPGN (Table 2). The patient was treated with diuretics and remission was achieved (Table 3).

Patient 2 is a 40-year-old previously healthy man who began to suffer from peripheral edema, low back pain and fatigue 1 month after acute NE. Proteinuria and hematuria were detected and renal biopsy revealed MPGN (Tables 1,2). Angiotensin-converting enzyme inhibitor was prescribed and the proteinuria gradually disappeared (Table 3).

Patient 3 is a 39-year-old woman with thyroxin substitution for hypothyroidism. One year before acute NE her urinary sediment had been intact. Five weeks after acute NE, peripheral edema, increase in weight and hypertension were noted, and urinalysis showed proteinuria and hematuria (Table 1). MPGN was diagnosed in renal biopsy (Table 2) (Figure 1). Diuretic therapy was started and the nephrotic syndrome went into remission (Table 3).

Patient 4 is a 48-year-old woman with thyroxin substitution for hypothyroidism. She had had no albuminuria 5 years before acute NE. During acute NE, serum creatinine rose to 306 µmol/l but after the polyuric phase fell to 89 µmol/l. Two weeks later she began to

suffer from diarrhea, fatigue and lack of appetite. She was hypertensive, serum creatinine rose to 233 µmol/l, and urinalysis revealed proteinuria and hematuria (Table 1). Renal biopsy showed MPGN (Table 2). Diuretic and anticoagulant therapies were started. After a follow-up of 1 week serum creatinine rose to 590 µmol/l. Steroid pulse therapy was initiated, followed by oral methylprednisolone. After 5 months serum creatinine was 190 µmol/l and proteinuria 1.3 g/d, and the steroid therapy was discontinued. Subsequently remission of the nephrotic syndrome was achieved (Table 3).

Patient 5 is a 59-year-old woman using hormone replacement therapy for menopausal symptoms. During acute NE, her serum creatinine concentration rose to 777 µmol/l. Three weeks later she began to experience dyspnea and cough. Proteinuria together with hematuria was detected (Table 1). Chest radiography showed pulmonary congestion and perfusion-ventilation lung scanning also pulmonary embolization. Renal biopsy revealed MPGN (Table 2). Serum creatinine increased from 94 µmol/l to 186 within 1 week and steroid therapy was initiated. The dose of steroid was gradually tapered and remission was achieved (Table 3).

Patient 6 is a previously healthy 27-year-old man whose serum creatinine rose to 541 µmol/l during acute NE, decreasing to 97
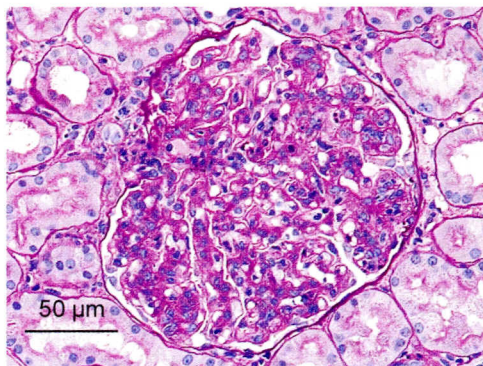
Figure 1a. Membranoproliferative glomerulo-nephritis in the renal biopsy specimen of Patient 3. Note accentuated lobulation together with increase of mesangial matrix and cellularity. Periodic acid-Schiff.
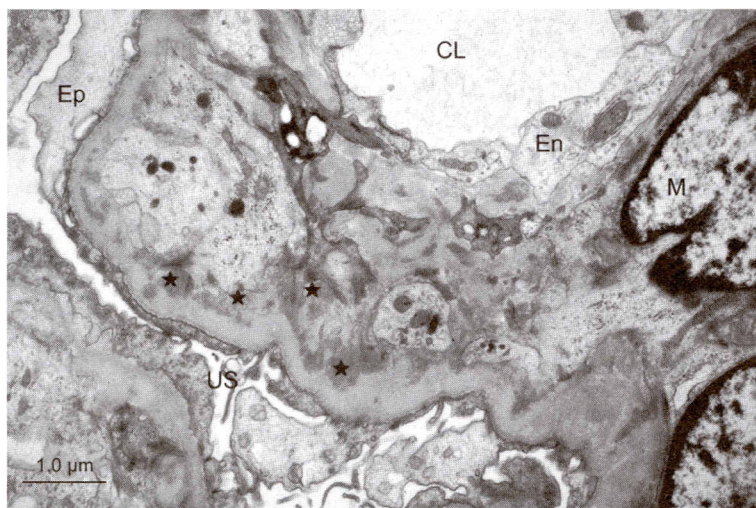

Figure 1b. Glomerular ultrastructure displaying mesangial interposition with electron-dense deposits (*). CL = capillary lumen; U = urinary space; En = endothelial cell; Ep = visceral epithelial cell; M = mesangial cell. Lead citrate and uranyl acetate.

Patient 7 is a 54-year-old man without previous medical history. Three weeks after acute NE, he noticed peripheral edema, felt shortness of breath and was hypertensive. Proteinuria concomitant with hematuria was detected (Table 1). Renal biopsy showed mesangial GN (MesGN) (Table 2). Angiotensin receptor blocker was prescribed for hypertension and remission was achieved (Table 3).

Clinical course of the patients

None of the patients had used antihypertensive medication before acute NE. All patients suffered from clinically typical NE, and a recent PUUV infection was serologically confirmed in all. The febrile phase during acute NE lasted for 5 – 8 days. The highest measured serum creatinine ranged from 50 to 777 µmol/l, the highest plasma C reactive protein from 8 to 149 mg/l, and the lowest blood platelet count from 32 to 159×10^9/l. At the end of the hospital treatment urine albumin dipstick tests of the patients ranged from negative to 1+.

One week to 3 months after discharge from hospital, the patients were admitted again due to rapidly emerged symptoms of nephrotic syndrome. Table 1 shows the clinical findings at the time of renal biopsy. All patients had nephrotic-range proteinuria concomitant with microscopic hematuria and hypoalbuminemia. Five were hypertensive. Renal biopsy specimens disclosed MPGN in Patients 1 – 5, MGN in Patient 6 and MesGN in Patient 7 as shown in Table 2. Tubuli and vessels were intact in all specimens. In IF, all glomerular findings were granular and diffuse in character. In patients with MPGN the renal biopsies were performed 4 – 5 weeks after recovery from acute NE. As shown in Table 3, all patients achieved remission during follow-up. The median time to remission was 0.6 years (range 0.5 – 5.5 y) and the median follow-up 1.7 years (0.7 – 15.6 y). In patients with MPGN, the median time to remission was 0.6 years (range 0.5 – 1.3 y).

µmol/l in 3 weeks. Three months later massive proteinuria (21.2 g/d) and hypoalbuminemia (13 g/l) were detected and the patient suffered from abdominal and chest pain. Venous thrombosis of the right kidney and pulmonary embolization were found. Renal biopsy was not performed due to anticoagulation therapy. Steroid therapy was subsequently initiated and after 4 months the proteinuria had diminished to 1.8 g and steroid was discontinued. However, 14 months after acute NE, the amount of proteinuria again increased to 21.5 g/d. Renal biopsy showed membranous GN (MGN) (Table 2). Remission was achieved 6 years after acute NE with one relapse of nephrotic syndrome 2 years thereafter (Table 3).

Discussion

We here describe 7 patients with GN emerging during the convalescent phase of serologically-confirmed PUU hantavirus infection. Five of them had MPGN, the other 2 patients MGN and MesGN, respectively.

The prognosis of MPGN after recent NE appears to be favorable. In our previous report on 5 patients with PUU virus-induced MPGN, remission of nephrotic syndrome was observed in 4 and only 1 entered end-stage renal failure [10]. In the present series, all 5 patients with MPGN achieved remission within a median time of only 0.6 years. One female patient (Patient 4) with MPGN had a clinical picture of rapidly progressive glomerulonephritis. She was treated with steroids, and remission was achieved rapidly. Naturally, we cannot exclude the possibility of remission being spontaneous. There were 2 other patients (Patients 1 and 5) who showed impairment of renal function at the time of renal biopsy. Renal biopsies of 2 of these 3 (Patients 4 and 5) revealed interstitial edema and biopsy of one (Patient 4) interstitial inflammatory cell infiltration in addition to typical morphological findings of MPGN, probably contributing to renal function. Furthermore, hemodynamic changes might have influenced the clinical course of these patients, because both diuretic therapy and nephrotic syndrome itself can predispose to relative hypovolemia and renal ischemia [15]. However, MPGN emerging after NE can also rapidly progress to end-stage renal disease, as we have previously described [10]. In the literature, the prognosis of MPGN is considered unfavorable. Spontaneous remission has been reported in only 2 – 20% of patients with MPGN [16] and 50 % either die or need renal replacement therapy 5 years after the renal biopsy [17].

In the present patients, nephrotic syndrome emerged shortly after a PUU virus infection, suggesting that viral infection might act as a trigger for glomerulonephrits. The three types of glomerulonephritis, MPGN, MGN and MesGN, described in the present patients have previously been reported in relation to hepatitis B and C (HCV) and human immunodeficiency viruses [18, 19]. A recent case report also suggests an association of Type I MPGN with Epstein-Barr virus infection [20], and MesGN has been linked to parvovirus B19 and Coxsackie virus B infections [19, 21].

The etiopathogenetic link between a viral infection and renal disease is usually difficult to establish [19]. The criteria for such causality should include recognition of clinical syndrome, serological diagnosis, identification of specific viral antigenemia, and the detection in glomerular structures of viral antigens and host antibodies [19]. Glomerular injury induced by viral infections may be the result of circulating immune complexes involving viral antigens and host antiviral antibodies [18] or endogenous antigens modified by viral injury and host auto-antibodies [22]. Furthermore, viral antigens may also bind to the glomerular structures by in situ immune-mediated mechanisms [23, 24], or viral proteins can induce an inflammatory renal disease [25, 26] or have a direct cytopathic effect [27].

Recently, some interesting data have been published about the possible role of toll-like receptors (TLRs) of innate immune system in immune complex-mediated glomerulonephritis. TLRs are activated by pathogen-associated danger signals and endogenous ligands, and may mediate glomerular injury by expression of chemokines and the deposition of immune complexes [28]. There are some data that Hantaan virus infection can induce the expression of TLR4 in vitro and enhance the production of cytokines [29]. TLRs may also contribute to the HCV-associated immune complex-mediated glomerulonephritis [30].

In the present report, patients developed nephrotic syndrome in the convalescent phase of an otherwise typical, serologically verified PUU hantavirus infection. PUU virus antigen was not sought in renal biopsies of the patients. Previous reports have provided evidence that in hantavirus pulmonary syndrome, hantavirus is present in the endothelial cells of interstitial capillaries of the medulla [31]. In HFRS there are some data that viral antigen is localized to the tubular epithelial cells [32, 33, 34]. There is no evidence of persistent hantavirus infection, and no clinical re-infections with PUUV or other human hantaviruses have ever been reported. Viral RNA can easily be detected within the first 9 days of symptomatic PUU virus infection, but convalescent samples (> 10 days after onset of symptoms) are consistently PCR-negative [35, 36, 37].

Physicians should be aware of the possibility of nephrotic syndrome emerging after recent NE. In such a case, renal biopsy most commonly reveals membranoproliferative glomerulonephritis (MPGN), but also other types such as membranous (MGN) and mesangial (MesGN) glomerulonephritides can occur. Puumala hantavirus-caused glomerulonephritis would appear to be an infrequent phenomenon, but the real incidence needs to be evaluated in a prospective study. The prognosis of glomerulonephritis caused by PUU hantavirus infection seems to be favorable and remission is usually achieved.

Acknowledgment

References

[1] Vapalahti O, Mustonen J, Lundkvist A, Henttonen H, Plyusnin A, Vaheri A. Hantavirus infections in Europe. Lancet Infect Dis. 2003; 3: 653-661.

[2] Muranyi W, Bahr U, Zeier M, van der Woude FJ. Hantavirus infection. J Am Soc Nephrol. 2005; 16: 3669-3679.

[3] Lähdevirta J. Nephropathia epidemica in Finland. A clinical histological and epidemiological study. Ann Clin Res. 1971; 3: 1-54.

[4] Mustonen J, Brummer-Korvenkontio M, Hedman K, Pasternack A, Pietilä K, Vaheri A. Nephropathia epidemica in Finland: a retrospective study of 126 cases. Scand J Infect Dis. 1994; 26: 7-13.

[5] Settergren B. Clinical aspects of nephropathia epidemica (Puumala virus infection) in Europe: a review. Scand J Infect Dis. 2000; 32: 125-132.

[6] Mustonen J, Helin H, Pietilä K, Brummer-Korvenkontio M, Hedman K, Vaheri A, Pasternack A. Renal biopsy findings and clinicopathologic correlations in nephropathia epidemica. Clin Nephrol. 1994; 41: 121-126.

[7] Ferluga D, A Vizjak. Hantavirus nephropathy. J Am Soc Nephrol. 2008; 19: 1653-1658.

[8] Miettinen MH, Mäkelä SM, Ala-Houhala IO, Huhtala HS, Kööbi T, Vaheri AI, Pasternack AI, Pörsti IH, Mustonen JT. Ten-year prognosis of Puumala hantavirus-induced acute interstitial nephritis. Kidney Int. 2006; 69: 2043-2048.

[9] Grcevska L, Polenakovic M, Oncevski A, Zografski D, Gligic A. Different pathohistological presentations of acute renal involvement in Hantaan virus infection: report of two cases. Clin Nephrol. 1990; 34: 197-201.

[10] Mustonen J, Mäkelä S, Helin H, Helanterä A, Miettinen M, Partanen J, Pasternack A. Mesangiocapillary glomerulonephritis caused by Puumala hantavirus infection. Nephron 2001; 89: 402-407.

[11] Vapalahti O, Lundkvist A, Kallio-Kokko H, Paukku K, Julkunen I, Lankinen H, Vaheri A. Antigenic properties and diagnostic potential of puumala virus nucleocapsid protein expressed in insect cells. J Clin Microbiol. 1996; 34: 119-125.

[12] Hujakka H, Koistinen V, Eerik,,inen P, Kuronen I, Mononen I, Parviainen M, Lundkvist A, Vaheri A, Närvänen A, Vapalahti O. New immunochromatographic rapid test for diagnosis of acute Puumala virus infection. J Clin Microbiol. 2001; 39: 2146-2150.

[13] Nolasco F, Cameron JS, Heywood EF, Hicks J, Ogg C, Williams DG. Adult-onset minimal change nephrotic syndrome: a long-term follow-up. Kidney Int. 1986; 29: 1215-1223.

[14] Waldman M, Crew RJ, Valeri A, Busch J, Stokes B, Markowitz G, D'Agati V, Appel G. Adult minimal-change disease: clinical characteristics, treatment, and outcomes. Clin J Am Soc Nephrol. 2007; 2: 445-453.

[15] Orth SR, Ritz E. The nephrotic syndrome. N Engl J Med. 1998; 338: 1202-1211.

[16] Williams DG. Mesangiocapillary glomerulonephritis. In: Davison AM, Cameron JS, Grungeld J-P, Kerr DNS, Ritz E, Winearls CG (eds). Oxford Textbook of Clinical Nephrology. Oxford: Oxford University Press; 1998. Vol 1: 591-612.

[17] Schmitt H, Bohle A, Reineke T, Mayer-Eichberger D, Vogl W. Long-term prognosis of membranoproliferative glomerulonephritis type I. Significance of clinical and morphological parameters: an investigation of 220 cases. Nephron. 1990; 55: 242-250.

[18] Glassock RJ. Immune complex-induced glomerular injury in viral diseases: an overview. Kidney Int. 1991; 35 (Suppl): 5-7.

[19] di Belgiojoso GB, Ferrario F, Landriani N. Virus-related glomerular diseases: histological and clinical aspects. J Nephrol. 2002; 15: 469-479.

[20] Karamadoukis L, Toth T, Tomson C. Membranoproliferative glomerulonephritis associated with an Epstein-Barr virus infection. NDT Plus. 2008; 5: 319-321.

[21] Conaldi PG, Biancone L, Bottelli A, De Martino A, Camussi G, Toniolo A. Distinct pathogenic effects of group B coxsackieviruses on human glomerular and tubular kidney cells. J Virol. 1997; 71: 9180-9187.

[22] Kimmel PL, Phillips TM. Immune complex glomerulonephritis associated with HIV infection. In: Kimmel PL, Berns JS, Stein JH (eds). Renal and urologic aspects of HIV infection, New York: Churchill Livingstone; 1995. p. 77-110.

[23] Golbus SM, Wilson CB. Experimental glomerulonephritis induced by in situ formation of immune complexes in glomerular capillary wall. Kidney Int. 1979; 16: 148-157.

[24] Couser WG. Mechanisms of glomerular injury in immune-complex disease. Kidney Int. 1985; 28: 569-583.

[25] Conaldi PG, Biancone L, Bottelli A, Wade-Evans A, Racusen LC, Boccellino M, Orlandi V, Serra C, Camussi G, Toniolo A. HIV-1 kills renal tubular ep-

ithelial cells in vitro by triggering an apoptotic pathway involving caspase activation and Fas upregulation. J Clin Invest. 1998; *102:* 2041-2049.

[26] *Segerer S, Nelson PJ, Schlondorff D.* Chemokines, chemokine receptors, and renal disease: from basic science to pathophysiologic and therapeutic studies. J Am Soc Nephrol. 2000; *11:* 152-176.

[27] *Glassock RJ, Cohen AH, Danovitch G, Parsa KP.* Human immunodeficiency virus (HIV) infection and the kidney. Ann Intern Med. 1990; *112:* 35-49.

[28] *Banas MC, Banas B, Hudkins KL, Wietecha TA, Iyoda M, Bock E, Hauser P, Pippin JW, Shankland SJ, Smith KD, Stoelcker B, Liu G, Gröne H-J, Krämer BK, Alpers CE.* TLR4 links podocytes with the innate immune system to mediate glomerular injury. J Am Soc Nephrol. 2008; *19:* 704-713.

[29] *Jiang H, Wang PZ, Zhang Y, Xu Z, Sun L, Wang LM, Huang CX, Lian JQ, Jia ZS, Li ZD, Bai XF.* Hantaan virus induces toll-like receptor 4 expression, leading to enhanced production of beta interferon, interleukin-6 and tumor necrosis factor-alpha. Virology. 2008; *380:* 52-59.

[30] *Wornle M, Schmid H, Banas B, Merkle M, Henger A, Roeder M, Blattner S, Bock E, Kretzler M, Gröne H-J, Schlöndorff D.* Novel role of toll-like receptor 3 in hepatitis C-associated glomerulonephritis. Am J Pathol. 2006; *168:* 370-385.

[31] *Zaki SR, Greer PW, Coffield LM, Goldsmith CS, Nolte KB, Foucar K, Feddersen RM, Zumwalt RE, Miller GL, Khan AS, Rollin PE, Ksiazek TG, Nichol ST, Mahy BWJ, Peters CJ.* Hantavirus pulmonary syndrome. Pathogenesis of an emerging infectious disease. Am J Pathol. 1995; *146:* 552-579.

[32] *Kim S, Kang ET, Kim YG, Han JS, Lee JS, Kim YI, Hall WC, Dalrymple JM, Peters CJ.* Localization of Hantaan viral envelope glycoproteins by monoclonal antibodies in renal tissues from patients with Korean hemorrhagic fever H. Am J Clin Pathol. 1993; *100:* 398-403.

[33] *Poljak M, Avsic Zupanc T.* Immunohistochemical detection of Hantaan virus antigen in renal tissue from patient with hemorrhagic fever with renal syndrome. Nephron. 1994; *67:* 252.

[34] *Groen J, Bruijn JA, Gerding MN, Jordans JGM, Moll van Charante AW, Osterhaus AD.* Hantavirus antigen detection in kidney biopsies from patients with nephropathia epidemica. Clin Nephrol. 1996; *46:* 379-383.

[35] *Hörling J, Lundkvist A, Persson K, Mullaart M, Dzagurova T, Dekonenko A, Tkachenko E, Niklasson B.* Detection and subsequent sequencing of Puumala virus from human specimens by PCR. J Clin Microbiol. 1995; *33:* 277-282.

[36] *Plyusnin A, Hörling J, Kanerva M, Mustonen J, Cheng Y, Partanen J, Vapalahti O, Kukkonen SK, Niemimaa J, Henttonen H, Niklasson B, Lundkvist A, Vaheri A.* Puumala hantavirus genome in patients with nephropathia epidemica: correlation of PCR positivity with HLA haplotype and link to viral sequences in local rodents. J Clin Microbiol. 1997; *35:* 1090-1096.

[37] *Plyusnin A, Mustonen J, Asikainen K, Plyusnina A, Niemimaa J, Henttonen H, Vaheri A.* Analysis of puumala hantavirus genome in patients with nephropathia epidemica and rodent carriers from the sites of infection. J Med Virol. 1999; *59:* 397-405.